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The BOTANICAL GAZETTE

FOUNDED BY JOHN M. COULTER

DECEMBER 1926

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Volume LXXXII Number 4

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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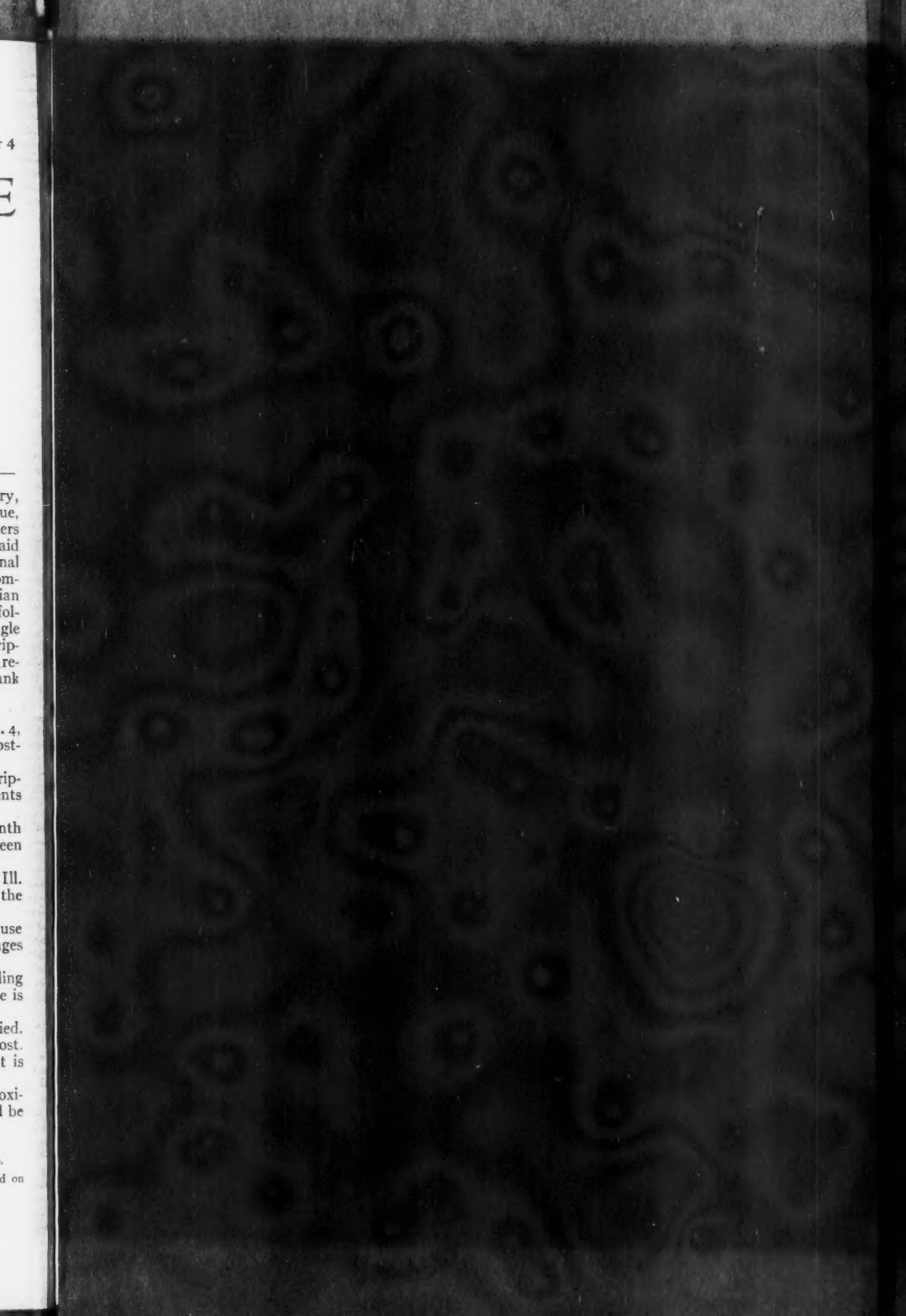

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December 1926 — January 1927

The World and Man

The advance of modern science has revealed a vast store of fascinating facts about the universe in which we live. More discoveries have been made in nearly every branch of science in the past two or three decades than in all the previous history of mankind. Scientists have disclosed a world of the infinitely small in electrons, atoms, and molecules; and of the enormously large in giant suns and galaxies of stars, billions of miles distant. They have looked back millions of years to the origins of worlds, and have traced their history through the geological ages to the present. Myriads of plants and animals have been studied, from the lowliest bacteria and protozoa up to man. Minute investigation of man himself—his origin, the wonders of his body, the development of his intelligence—has yielded astonishing facts. Inanimate matter has revealed marvelous secrets to the modern chemist. The physicist has explained the forces we see about us every day, and the biologist is seeking to explain the very secret of life itself.

To help the inquiring reader in his efforts to keep up with the procession of modern science, sixteen members of the Faculty of the University of Chicago have written *The Nature of the World and of Man*, which E. E. Slosson describes as a book for "those who have not been to college and those who have."

These men, specialists in their fields, are the authors: Forest Ray Moulton, Rollin T. Chamberlin, J Harlen Bretz, Harvey B. Lemon, Julius Stieglitz, Horatio Hackett Newman (the editor), Edwin Oakes Jordan, Merle C. Coulter, Henry Chandler Cowles, Warder C. Allee, Alfred S. Romer, Fay-Cooper Cole, Elliot R. Downing, George W. Bartelmez, Anton J. Carlson, and Charles Hubbard Judd.

Beginning with the outstanding facts about the solar system, each of the sixteen authors has described that phase of development with which he is most familiar: the origin and early stages of the earth; geological processes and the earth's history; the nature of energy and matter; bacteria and their origin; evolution of the plant kingdom, the vertebrates, and the invertebrates; interactions between plants and their environment; the coming of man; organic evolution and the origin of life; human inheritance; structure of man; the living process; and mind in evolution.

Throughout, the authors have emphasized the conception of science as a glowing, living thing inextricably bound up with every human life. With all its multiplicity of authorship, the book has (in the words of the *Chicago Tribune*) "taken on the unity, the coherence, the march of one great epic poem."

Stuart Pratt Sherman said not long ago: "I have a strong impression that our 'average' American is waking up, responding to the new emphasis on 'adult education,' becoming infatuated with the idea of having his education go on as long as he does. He has got wind of the secret that in all the fields of knowledge enormous changes and advances have been made in the last twenty-five years and are still being made at this present hour. His sporting desire to keep up with his times grows keener and the range of his curiosity will soon be encyclopedic."

The popularity of *The Nature of the World and of Man* is evidence of the awakening of the adult public to the possibilities of

further education. For the reader outside the universities the book is a guide to the world of contemporary science; it enables him to form a conception of how the earth came to be, what its place is in the vast physical universe, how life originated; it gives him glimpses of the monsters of early ages; shows him how Cro-Magnon man lived, how atoms are held together in a molecule, how invisible throngs of bacteria surround us, how life came from the sea to the land, why insanity is increasing, how speech developed, how hormones regulate bodily processes. . . . .

The Nature of the World and of Man contains the subject matter of a "survey course" given each year by its authors to a selected group of beginning students at the University of Chicago. The success of the experiment upon which it is based suggests a wide use of this text in similar courses in other institutions to give the beginner a preliminary view of the surrounding world and of his possible function in it.

In this day of increasing specialization and a confusingly large body of human knowledge, orientation toward the more general aspects of the world and of man is a necessity. In the belief of the authors of this text, orientation may best begin with a presentation of what modern science holds as truth. They have therefore attempted to give the student that acquaintance with the general outlines of scientific thought which is the ideal point of departure for his entire intellectual development, and which alone affords him the proper perspective on the world of modern thought. More specialized courses in thinking, man in society, or the arts, may well follow, but this course, being more informational, more inclusive, and easier for the beginner should be the point of departure. *THE NATURE OF THE WORLD AND OF MAN*. By SIXTEEN MEMBERS OF THE FACULTY OF THE UNIVERSITY OF CHICAGO. *$4.00, postpaid $4.15.*

* * *

Current American Prose

In a volume of provocative essays, *The Outlook for American Prose*, Joseph Warren Beach searches the current prose of reflection, reminiscence, criticism, and social commentary for auguries of a first-rate style. He judges as stylists the American prose artists who are now in the public eye. Timely and incisive comment from this candid and erudite observer will be not unwelcome to readers who have become aware of the development of American prose style in such books as *Troubadour, Straws and Prayer-Books*, and *A Story-Teller's Story*.

A professed eclectic, and adherent of no particular school or tendency, Mr. Beach seeks the perfect style wherever it may be found. Looking about for American writers of cultivated philosophical prose to compare with Bertrand Russell and Havelock Ellis; biographers to rank with Lytton Strachey; newspaper essayists to place beside Chesterton; and critics as stimulating as Shaw, he advances a gratifying number of claimants to distinction. In Sherwood Anderson, James Branch Cabell, H. L. Mencken, Stuart Pratt Sherman, Paul Rosenfeld, Simeon Strunsky, and Alfred Kreymborg, Mr. Beach finds the most hopeful signs of a distinguished prose art in America, and whether or not you agree with him, you will have to reckon with his arresting dicta on the luminaries of the day.

"Old fashioned or new fashioned, what we want is something first rate," says Mr. Beach. His regard for stylistic perfection leads him to protest against the incoherence of Hergesheimer; the lack of precision in John Dewey; the naïveté of Dreiser; the scientific jargon of Van Wyck Brooks; and the affectations of Van Vechten. Sentimental violence and overuse of such words as "devastating" and "poignant" are charged against Ben Hecht, Hutchinson, Walpole, and Hergesheimer.

SHERWOOD ANDERSON

—"*A prose-writer who dares to use the devices of the poet.*"

Mr. Beach finds Gamaliel Bradford too serious minded and turns for lightness of touch to Strunsky and Morley. In Anderson, Cabell, Mencken, and Sherman, he finds the supreme qualities of flavor and individuality, gusto, and precision. Kreymborg's style is "uniformly graceful, delicately tinted."

VAN VECHTEN

—"*Baedeker of the intelligentsia.*"

Mr. Beach has put to good use a conception of the genuine critical spirit characterized by candor and tolerance. He admits that he has been denied that arrogance which enables a critic to tell at a glance whether or not a writer is one of the immortals. First-rate prose he finds most frequently in the unpretentious styles that slip in between rigid categories.

You will want to read this book for the vigor of its outlook upon American prose and for its timely comment on some of the most interesting figures in modern literature.

H. L. Mencken says in the *American Mercury:* "This is perhaps the first book, written by an American, in which precept is backed up by practice. Mr. Beach writes very effectively, and has much of interest to say. His judgments on the writing of Dreiser, Anderson, Van Vechten and other living Americans are well-informed and plausible." *THE OUTLOOK FOR AMERICAN PROSE.* By Joseph Warren Beach. *$2.50, postpaid $2.60.*

* * *

New Version of the Psalms

For a long time we have needed a translation of the Book of Psalms into the language of our day—a translation that would give the modern reader new insight into the classical rendering of the King James Version, and enable him to see in the Psalms the meaning they originally had. Such a translation is the new version by J. M. Powis Smith, which removes the stumbling-blocks of a centuries-old vocabulary, and divests the verses of unwarranted meanings which a succession of early translators read into them. This version is based upon the official Hebrew text, corrected and emended in accordance with the findings of present-day scholarship.

Dr. Smith expresses at the outset his admiration for the King James Version of the Psalms as "a masterpiece of English literature, unrivalled in beauty," and disclaims any attempt to supplant it with his own version. His purpose is rather to express, as completely and as accurately as the limitations of language permit, the thought and feeling of the original; and to incorporate the scholarship of recent decades, with its more thoroughgoing text-critical method and improved philological results. His aim has been at all times to create a clear and uncorrupted version in which the reader of today will be able to see unhampered the beauties and true meaning of the world's greatest hymnbook.

This translation is intended for the public rather than for technical study; it is not, however, an attempt to bring the Psalms down to the level of the mythical "man on the street." The Psalms are here reproduced in a language and style befitting their spirit and content.

While seeking to avoid a bald literalism, Dr. Smith has preferred to err on that side rather than upon the side of paraphrastic expansion. His aim being always accuracy of meaning, he has chosen not to take the liberties of paraphrastic rendering.

The translator brings to his task unusual qualifications. He is professor of Semitic languages and literatures in the University of Chicago, editor of the *American Journal of Semitic Languages and Literatures*, and one of the foremost Hebrew scholars in this country. He is the author of several well-known books on Old Testament subjects.

This version of the Psalms is the first step in the complete American translation of the Old Testament, upon which a committee of Hebrew scholars is now working and which will require a year for its completion.

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Brains of Rats and Men

By C. JUDSON HERRICK

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A new volume, *The Urban Community*, edited by E. W. Burgess, and a previously published one, *The City*, by Robert E. Park, E. W. Burgess, and others, form together a much-needed introduction to an urban sociology. They are the nearest approach thus far to textbooks in urban sociology, including under that term community organization, urban geography, and human ecology. Based upon a conception of the city as a living organism, they are the first steps in an attempt to supply that basic understanding of the city needed to unify the many present-day urban movements. These two books offer an introduction, not merely to the facts of urban life, but to the processes involved in human life from the standpoint of environment.

Continuing the work of outlining the field of urban sociology as begun in *The City*, the new volume, *The Urban Community*, is a prospectus of the present state and promise of sociological research in this field. It indicates and illustrates for students of the subject the different techniques of research, ecological, cultural, and statistical, which are being applied to the study of the city.

It covers a wide range of phenomena from human nature and personality to statistics of city life. It deals with fundamental problems and contains more modern applications of the subject and more statistics on urban problems than any other volume. It will be a most helpful text for teachers of urban sociology and community organization, and will admirably aid all students of these subjects, as well as those interested in urban geography.

The chapters in this volume are selections from the proceedings of the 1925 meeting of the American Sociological Society.

*

In *The City* a group of sociologists brought to the study of urban problems a technique

which assured a more searching and disinterested analysis than had previously been attained. This pioneer work is concerned with outlining a program for future investigation of human nature and social life under modern urban conditions.

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THE

BOTANICAL GAZETTE

December 1926

DISTRIBUTION OF NATIVE PLANTS AND WEEDS ON CERTAIN SOIL TYPES IN EASTERN TEXAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 359

S. R. WARNER

Introduction

No ecologist in traveling through eastern and central Texas could fail to notice how closely plant formations follow certain types of soil in this region. This is strikingly expressed in the formation types of prairie, post oak ridges, and pine land. The entire upland vegetation of the inner coastal plains province is apparently an edaphic complex. So insistent is the nature of the soil in determining the distribution of the vegetation of this region, that one feels that he could assume that all the upland vegetation of this latitude in the inner coastal plains east to Georgia is prairie or forest, depending on the type of soil.

In a study of the correlation between vegetation and certain soil types an area is used on which a soil survey has been made. The soils considered are the most extensive and distinctive in the inner coastal plains province of the southern United States. The writer has attempted to make an analysis of the factors that are dominant in each soil, to define the composition of each edaphic formation, to discover possible stages in plant succession, and to point out the indicator value of certain species and communities. The region of this study may be of general interest because of its age. The prairie soils have never been glaciated, and the sandy soils are leached residues of quartz and silicates, and not mixtures of mineral fragments such

as those usually considered in studies of the upper Mississippi valley and Rocky Mountain regions. Eastern Texas, because of its humidity, affords an excellent opportunity for the observation of other factors than water in determining plant associations.

The literature bearing on the factors determining prairie and forest formations and tension zones is too extensive to be reviewed in the short space of this article. The literature that relates to the use of soil types as a basis for defining plant communities is quite limited. FULLER (10) has pointed out that the forests in the prairie region of Illinois follow certain soil types, and KELLY (11) has considered plants as indicators of soil types in Pennsylvania. A survey of the climatic associations of Texas east of the 98th meridian has recently been made by THARP (17). An ecological survey of the entire state was made earlier by BRAY (1). FOSTER, KRAUSZ, and LEIDIGH (6) made a survey of Texas woodlands. CAMPBELL (2) made a survey of the plant associations in Brazos County, Texas. Several local surveys have been made throughout the area, but the writer has no knowledge of any attempts in Texas to use edaphic conditions as the basis for the formations of plant associations. The classification of plant communities follows the terms defined by NICHOLS (13, 14), the taxonomic nomenclature is based on that of SMALL (16), and dynamic concepts of vegetation are based on the work of COWLES (5) and CLEMENTS (3).

Location and description of soils

In the inner division of the Atlantic and Gulf coastal plains soil province of the southern United States there exist in the uplands two characteristic soil groups differing as to maturity, the leached soils and the calcareous soils. The first group is probably derived from the Piedmont Appalachian and Ozark material, and because of the long weathering of the minerals of these soils they may be considered as old or mature, both as to surface and subsoil. The second group is derived from limestone and chalk, probably transported from western prairies, and consists of unleached soils high in lime and organic content and low in sand. They are very subject to erosion, and it is partly for this reason that they continue their youth, because they are constantly exposing new limy soils from beneath.

The following is a descriptive key, adapted from MARBUT (12), to the soil series of this province which are included in this study:

A. Piedmont Appalachian material.
  I. Soils with friable subsoils, drainage well established.
    1. Gray soils, subsoil yellow..... ..Norfolk series
    2. Gray soils, subsoil yellow, iron concretions.....Tifton series
    3. Gray soils, subsoil light red to reddish yellow.....Ruston series
    4. Gray soils, subsoil dull red to normal red.....Orangeburg series
  II. Soils with plastic subsoil, drainage well established.
    1. Gray soils (reddish gray) subsoil mottled red, yellow.....Susquehanna series
B. Calcareous material.
  I. Soils with plastic subsoils, drainage well established.
    1. Black soil, subsoil light gray to light brown, highly calcareous.....Houston series

The soils with friable subsoils include four closely related series which differ largely on the basis of color, resulting from the effects of various degrees of weathering on the iron content. The floristic difference of these soils is small and the vegetation will be treated as one association-complex. The Norfolk soil will be considered the type for this group.

The portion chosen for intensive study was the area of the Willis soil survey in Montgomery County, Texas, which is 40 miles north of Houston with a latitude of 30° 30′ and a longitude of 95° 30′. This area is situated just west of the long-leaf pine forest, and is designated by FOSTER (6) as the *Pinus taeda* region, and by THARP (17) as the *Pinus-Quercus* ecotone zone. Reconnoissance studies were made by the writer over much of the area in Texas bounded by longitude meridians 95 and 98, and latitudes 30 and 32. Some study was made of the most easterly extension of the Houston soil in Montgomery County, Alabama, and of the western extension of the Norfolk soils in Bastrop County, Texas. The Alabama prairie occurs on Houston soils about 600 miles east of the central Texas prairies, likewise on Houston soils. The Bastrop area is a disjunct pine forest

80 miles west of other pines to the east, and 120 miles west of the Willis area. Some study was made of the vegetation occurring on the calcareous sands of the eastern coastal strand of Texas. The Willis area contains the dominant soil series of the southern United States in the inner coastal plains province. The most extensive soils of the survey belong to the Norfolk and Houston series. Small areas of Susquehanna, Orangeburg, Lufkin, and Tifton soils occur in this region. The plant associations of these soils were followed into adjoining counties. This study extended over a period of six years, concluding in the year 1925.

Factors

The edaphic formation types seem to be determined by soil texture and aeration, water, and chemical and biotic factors. Other factors of temperature, topography, and transpiration are very important in influencing the communities present within each edaphic formation, but they are not generally applicable in defining the formations of the respective soil types.

SOIL TEXTURE AND AERATION.—The soil structure in itself is an important factor in determining plant distribution. Heavy clays and chalky soils impede root elongation and may influence the development of root hairs. The greatest influence of soil texture is indirectly related to aeration, water capacity, and water movement. Texturally the soil types of this area fall into five classes: (1) the fine sand with fine sand subsoils which have the lowest water capacity and best aeration; (2) the fine sandy loams with friable sand-clay subsoil which permit good aeration, and especially large water capacity in the subsoils; (3) the fine sandy loam with plastic clay subsoils which permit only slow penetration and are poorly aerated in the subsoil; (4) Susquehanna clays with plastic subsoils which possess a high water capacity but puddle badly and lose water rapidly in drought; (5) Houston black clay, with clay surface and subsoil, which crumbles and cracks on drying, and permits ready saturation of the surface but resists percolation and the movement of water and air in the substratum after saturation.

It should be emphasized that when Houston black clay is very wet it is a water-logged soil, and the impervious character of the

chalk and heavy clay of the subsoil so checks the downward movement of water as largely to prevent any movement of oxygen except by diffusion. The poor oxygen supply to the deeper substratum during most of the winter and spring months, when these soils are saturated, is sufficient to act unfavorably on the deep rooted development of many plants. The writer feels that a deficiency of oxygen in the subsoil is one of the operating forces to exclude trees; however, no experimental data were obtained on this point. The difficulty of root penetration in Houston clay is seen in the tendency of the smaller roots to follow cleavage planes, which are less developed in the deeper subsoil. This characteristic itself may be a factor in restricting deep root development.

There is probably a detrimental effect resulting from cracking that may be of ecological significance. The Houston soils, on account of their large water-holding capacity and high content of clay and other colloidal matter, are very subject to checking or cracking. These cracks may reach a depth of more than 3 feet. Lateral roots are normally broken by this shrinkage. The writer observed roots of *Pinus echinata* 0.5 cm. in diameter which were broken in this way. Much greater tensile strength was exhibited by roots of *Toxylon pomiferum* and *Celtis mississippiensis*. Thus it might be said that, since cracking is unfavorable to lateral roots and aeration is unfavorable to deep roots, conditions tend to favor small descending types of root systems. These soils crack before the wilting coefficient is reached. Blocks of soil are sufficiently large to accommodate the root system of most grasses.

Table I summarizes the mechanical analyses of twenty-seven soil samples of eastern and central Texas counties. This table and the foregoing descriptions of the soil types give the best picture of the texture and probable aeration of the respective soils.

WATER.—The soils of the regions under consideration are normally supplied with an adequate rainfall. The averages are: Willis, Texas, 45 inches; Bastrop, Texas, 35 inches; and Montgomery County, Alabama, 51 inches. The rainfall evaporation ratio of 90 per cent for Alabama and Willis, Texas, is well above the 80 per cent emphasized by SAMPSON (15) as the climatic limit of prairies. The rainfall in the year from June 1924 to July 1925 was very light:

Willis 13.35 inches; Bastrop 13.39 inches; and Montgomery 37.5 inches (24 inches of this fell during December, January, February, and March). This is evidence that at times there may be extreme departure from the average when the water reserve of the soil becomes an important factor in the life of vegetation. Determinations of the amount of water in the respective soils at the end of drought periods are shown in table II.

TABLE I

SUMMARY OF AVAILABLE MECHANICAL ANALYSES OF CERTAIN SOILS IN EASTERN TEXAS*

Soil	No. of samples	Coarse and medium sand		Fine sand		Very fine sand		Total surface sand	Total subsoil sand	Silt		Clay		Total surface silt and clay	Total silt and clay subsoil
		Soil	Subsoil	Soil	Subsoil	Soil	Subsoil			Soil	Subsoil	Soil	Subsoil		
Norfolk fine sand	5	4.7	5.38	62.4	62.6	18.1	16.7	85.2	84.6	12.	11.5	2.8	3.8	14.8	15.4
Norfolk fine sandy loam	5	0.63	0.6	31.5	31.	39.3	26.1	71.4	57.7	23.1	19.9	4.96	21.7	28.1	41.6
Orangeburg fine sandy loam	2	2.6	2.2	35.4	21.2	24.2	22.4	62.2	45.8	31.2	29.2	6.4	24.	37.6	53.2
Susquehanna fine sandy loam	6	1.45	.85	35.6	27.2	27.3	16.9	64.4	44.9	24.9	17.9	8.2	37.0	33.1	54.9
Susquehanna clay	3	2.6	1.2	20.5	9.1	26.8	13.3	49.9	23.6	38.2	25.5	12.4	49.8	50.6	76.3
Houston clay	6	1.7	1.9	7.9	5.8	11.0	8.0	20.6	15.7	49.1	50.2	30.0	33.2	79.1	83.4

* Figures compiled from numerous reports of soil surveys of Texas counties by the United States Bureau of Soils.

From these figures it is evident that the amount of available water at a depth of 24 inches in the uncultivated Houston soil, the prairie type, is as great as it is in the other uncultivated soils at this depth, and none of these soils possess available water at a depth of 12 inches. These figures also indicate that cultivated Houston black clay has a greater water retaining capacity than the Norfolk and Susquehanna soils. This fact is supported by observations of crops under cultivation on the soil types during prolonged dry periods. Especially is this noticeable when comparison is made between Houston soils and the tight silty post oak soils. The greater water retaining capacity characteristic of the Houston

soils is associated with the high content and greater depth of organic matter, the texture, and the flocculent character of the soil. The presence of lime in these soils aids in the retention of soil water by causing the soil surface to crumble on drying, thus forming a mulch. The severe cracking or checking of this soil in drought periods is an important influence in water loss, but its importance is usually over estimated. The cracking does not indicate that the soil is necessarily drier but that it shrinks on drying. It is because of these characteristics that summer rains are readily absorbed with little or no run-off. The reaction of vegetation to drought in prairies of the

TABLE II

WATER IN SOIL IN AUGUST AT END OF 60-DAY DROUGHT PERIOD

	NORFOLK FINE SAND		NORFOLK FINE SANDY LOAM		SUSQUEHANNA RED CLAY		HOUSTON BLACK CLAY	
	Depth of sample (percentage)							
	12 in.	24 in.	12 in.	24 in.	12 in.	24 in.	12 in.	24 in.
Wilting point*.....	1–2	1–2	3.1	3.1	10.3	10.3	16.1	16.1
Pasture sod								
Total water.....	1.8	2.1	2.8	5.5	9.5	13.6	14.5	20.2
Available water.....	0.0	Trace	0.0	2.4	0.0	3.3	0.0	4.1
Cultivated soil								
Total water.....	3.3	4.2	6.0	11.4	1.1	21.0	30.7	34.8
Available water.....	1.3	2.2	2.9	8.3	2.0	11.0	14.6	18.7

* Wilting point determined on basis of hygroscopic moisture.

Houston clay seems to depend more on the character and extent of root systems of the plant and movement of water in the soils than on their water content. The tree growth forms apparently find the Houston soils more xerophytic than the fine sandy loams; this is true at least in the late summer and fall months during a prolonged drought. The other soils, which show xerophytism, usually manifest this condition early in the season on herbaceous growth forms. The four most xerophytic habitats are the ridges of deep Norfolk sand, the eroded slopes of Susquehanna clay, the shallow chalky phases of the Houston soils, and the silty clay transitional soils (Lufkin type) between true prairie and true woodland.

The following observations may best express the reaction of the vegetation to the water factor in the drought period of 1924–1925.

In the xerophytic sandy situations there was little dying of larger pines, but there was a high fatality among pines under five years of age and in upland hardwoods, as *Quercus digitata*, *Q. marylandica*, and *Hicoria glabra*. In the margins of the Houston soil there was considerable dying of pines of all ages, also of *Quercus digitata*, *Q. marylandica*, *Hicoria villosa*, *Crataegus viridis*, and *C. spathulata*, but little effect was shown on *Q. minor*, *Celtis mississippiensis*, *Toxylon pomiferum*, and *Ulmus crassifolia*. The apparent reason for the greater drought tolerance of pines in Norfolk sand than in Houston soils is because the loose texture of Norfolk soil is favorable to the inherent tendency of *Pinus taeda* and *P. echinata* to form deep extensive root systems. In the transitional zone between prairie and forest there occurred the greatest fatality of tree growth forms. This was especially noticeable among older trees. At many places in this zone as high as 20 per cent of the pines were lost. Almost as great a loss occurred in *Q. digitata*. It is interesting to note that those species which showed the greatest drought tolerance were the ones showing the greatest defoliation, namely, *Q. minor*, *Celtis mississippiensis*, *Ulmus crassifolia*, *Toxylon pomiferum*, *Populus deltoides*, and *Morus rubra*.

Topography and transpiration are important factors in determining the water relations of a community, but since the edaphic formations of this region occur within a few yards of each other and occupy equally exposed situations, it seems unjustifiable to consider either of them of importance in determining the edaphic formations under consideration. All prairies of this area occur on upland Houston soil and are the result of characteristics possessed by this soil. There is little apparent relation between their occurrence and the direction of the slope or the position of exposure. Small prairies sometimes exist on northern slopes and are surrounded by virgin forest. Some of these show only a few yards of forest invasion in a period of many centuries.

That water is an important factor is shown by the xerophytic, semixerophytic, mesophytic, and hydrophytic divisions of edaphic formations. Since these parallel communities are not similar in leached and unleached soils, water apparently is not the controlling factor in determining the difference in the structure of edaphic

formations under consideration. This is most strikingly demonstrated by the dissimilarity in the societies of winter annuals and weeds on cultivated soils which are little affected by the water differences. The conditions of the leached upland ridges apparently are more xerophytic than those of the exposed prairies. The vegetation of the former shows a higher fatality than that of the latter; therefore neither transpiration nor soil water explains adequately the difference in the structure of these communities.

CHEMICAL FACTORS.—The leached and unleached soils which support unlike plant formations show marked chemical differences. These chemical differences have been investigated from the standpoint of soil reaction with regard to the hydrogen-ion concentration and amount of titratable acid, and from the standpoint of composition of the soil, with especial consideration given its calcium carbonate content. The colorimetric method recommended by WHERRY (21) has been followed in determining the P_H, and the results are summarized in table III. The majority of these determinations were made during the drought period of 1924–1925. The P_H of the leached soils is seen to be characteristically acid; that of the unleached Houston soil is alkaline. The acidity seems to be of two origins. One is associated with surface organic matter, as in woods where it is greatest near the surface and decreases with the depth of the soil in soils containing little clay. The other type of acidity is associated with the mineral constituents of the soil. It increases proportionately with the clay content to a depth of about 10 inches; below this the substratum becomes less acid. This indicates that the acidity is probably related to the accumulation of weathered aluminum and iron silicates at this depth. In the soils low in organic matter, as in sterile old fields, the deep Norfolk fine sand was nearly neutral, due to the almost negative action of the sand; the fine sandy loams were more acid; and the Susquehanna soils highly acid, due to the influence of increased mineral acids from the content of clay. The poorest growth of vegetation occurs upon these leached and acid slopes. The slopes of the Norfolk soil show a greater acidity than its more level areas, due to the effects of leaching; the slopes of the Houston clay are more alkaline than its level areas, due to the loss of surface soil by erosion.

The difference in the influence of carbon dioxide on roots in acid and in alkaline soils may have some influence on plant distribution.

In leached soils carbon dioxide is always present as carbonic acid acting upon the roots and the soil. This is shown in the acidity of spring water in these areas. Examination of ten such springs gave

TABLE III

SOIL REACTION EXPRESSED IN POTENTIAL HYDROGEN

FORMATION TYPE	SURFACE SOIL				SUBSOIL	
	Low	High	Average	Character	10 in.	3 ft.
Willis area						
Houston black clay						
Xerophytic	7.5	9.0	8.5	8.5	8.5	8.5
Semixerophytic	7.0	8.5	8.0	8.0	8.5	8.5
Mesophytic	6.0	8.0	7.4	7.2	7.5	8.0
Hydro-mesophytic	7.0	8.0	7.5	7.5	7.5	.....
Susquehanna reddish clay						
Xerophytic	4.8	6.5	5.4	5.5	5.2	5.5
Norfolk fine sandy loam						
Semixerophytic woods	5.2	7	5.8	5.8	5.5	5.8
Semixerophytic cultivated	5.4	7.2	6.2	6.2	5.8	5.8
Semixerophytic sterile old fields	5.0	6.5	5.6	5.5	5.2	5.5
Mesophytic	5.2	7.2	5.8	5.8	6.0	6.0
Hydro-mesophytic seepy slopes	4.8	6.0	5.2	5.0	5.2	.....
Norfolk deep fine sand						
Xerophytic woods	4.8	7.0	6.1	6.0	6.4	6.5
Xerophytic new ground	5.8	7.5	6.6	6.8	6.5	6.5
Xerophytic sterile old fields	6.0	7.0	6.5	6.5	6.2	6.5
Bastrop area						
Susquehanna gravelly loam	4.5	7.0	5.5	5.2	5.0	5.0
Norfolk fine sand	5.2	7.5	6.3	6.4	5.7	5.8
Other areas						
Beach strand (marly sands)	7.5	9.0	8.5	8.5	8.5	.....
Orangeburg fine sandy loam	5.4	6.8	6.2	6.0	5.8	6.5
Peaty mucks	4.2	4.8	4.5	4.5	4.5	.....
Alluvial soils	5.5	8.5	7.2	7.0	7.4	7.0

an average acidity of P_H 5.4, which could be destroyed by boiling. In Houston soils the acid action of excreted carbon dioxide is quickly lost by its combination with the calcium carbonate to form a bicarbonate. If carbon dioxide can have any influence on plant distribution, therefore, it should have this effect in leached and limy soils where it occurs in different conditions.

The Norfolk and Susquehanna soils were tested by the VEITCH (18) method for specific acidity. Five tests were made for each soil,

and every analysis of the uplands of these two soils showed an absorption of the base by the soil. The acidity in parts per million of the Susquehanna soil ranged from 400 to 2100; that of the Norfolk sand ranged from 50 to 500; and that of the Norfolk fine sandy loam ranged from 0 to 700.

The Houston soils are rich in lime. Ninety-nine samples out of every hundred taken at random give an active effervescence when treated with hydrochloric acid. Quantitative determinations from three typical fields of this soil type gave the following percentages of

TABLE IV

CHEMICAL CONSTITUENTS OF CERTAIN SOIL TYPES IN EASTERN TEXAS

Soils	No. of samples	Phosphoric acid	Nitrogen	Potash	Lime	Magnesia	Ignition loss	No. samples showing acidity	Average acidity of acid samples
Norfolk sand and fine sand..	9	0.02	0.04	0.15	0.13	0.11	1.99	2	250
Norfolk fine sandy loam.....	7	0.02	0.03	0.09	0.12	0.07	1.25	7	309
Orangeburg sand.....	1	0.02	.02	.15	.05	.09	0.17	1	200
Orangeburg fine sand.....	1	0.01	.02	.07	.14	.14	0.94	1	300
Orangeburg fine sandy loam.	2	0.02	.02	.10	.10	.08	1.26	2	325
Susquehanna fine sand.....	2	0.02	.02	.10	.05	.07	1.08	2	450
Susquehanna fine sandy loam.	5	0.02	.03	.16	.09	.10	1.80	3	660
Ruston fine sandy loam.....	2	0.02	.03	.10	0.14	0.07	1.66	1	600
Houston black clay.....	5	0.18	.12	.49	7.6	1.15	8.25	0	0
Houston black clay.....	5	0.06	.10	.55	.84	.78	5.27	1	50
Susquehanna clay.....	1	0.07	0.13	0.44	0.26	0.49	7.68	1	330

CaO: surface soil 4.28, 4.06, and 5.02; subsoil 10.24, 7.63, and 12.48. Similar analyses of the more calcareous spots gave a CaO content of 7.75 per cent for the surface and 13.63 per cent for the subsoil.

These determinations show high base content in Houston soils, and a general deficiency of bases in the Susquehanna and Norfolk soils. That similar conditions exist for these respective soils throughout eastern Texas is shown in table IV, which is a summary of analyses of these soils made by FRAPS (**7, 8, 9**). The close correlation between soils of high basic content and the occurrence of prairie species, which exist in edaphic formations of this region, holds true for the climatic prairies to the west. A similar correlation between edaphic formations of leached soils in this region exists for

the climatic forest to the east. In a compilation of averages from COFFEY (4), the prairie states of Nebraska, Kansas, and Iowa showed an average analysis of potash 0.45, phosphoric acid 0.22, lime 0.80, and magnesia 0.32. The southeastern timbered states of North Carolina, South Carolina, and Georgia show potash 0.12, phosphoric acid 0.08, lime 0.13, and magnesia 0.11. These figures indicate that the chemical nature of the soil may be as important a factor in determining the vegetation of climatic formations as in determining edaphic formations.

BIOTIC FACTORS.—That microorganisms may play an important rôle in developing soil characteristics and plant associations is quite

TABLE V

NUMBER OF MICROORGANISMS PER GRAM IN PASTURE SOIL FOR MEDIA AND DETERMINATIONS*

	NORFOLK FINE SANDY LOAM PH 5.8	SUSQUEHANNA RED CLAY PH 5.2	HOUSTON BLACK CLAY PH 8
Bacteria			
Media PH 7	8,600,000	3,400,000	14,800,000
Media PH 5.4	7,700,000	3,700,000	9,300,000
Actinomycetes	24 per cent	10 per cent	37 per cent
Bacteria	76 per cent	90 per cent	63 per cent
Soil fungi	63,000	44,300	14,400

* WAKSMAN and FRED'S (19) methods.

apparent, but this factor is difficult of analysis. The development of black humus and black soils in the presence of lime and the development of gray soils in a deficiency of lime are probably associated with the activity of soil organisms. WAKSMAN (20) concludes that the greater humus in black soil is due to greater carbon residue from bacterial action, since bacteria use carbon less effectively than fungi. The two groups of soils contrasted in this study are respectively gray and black; therefore, some analysis of this factor becomes necessary (table V).

The observations made from the study of microorganisms may be summarized as follows: (1) Bacteria are more numerous in unleached black soils and fungi are more abundant in gray leached soils. (2) Many bacteria of the black soils which will develop in neutral or alkaline media will not develop in this same medium which has had its acidity increased to a point comparable with the

acidity existing in gray soils. (3) The fungi of the two types of soil show quantitative and qualitative differences, the latter being perhaps of more importance; these the writer hopes to treat in a later paper. (4) Since it is true that the distribution of many microorganisms is dependent on certain chemical conditions, it is reasonable to assume that many seed plants are influenced by such conditions. This relation may have a direct influence in the rôle of parasitism, symbiosis, and the favorable toxic products of microorganisms.

Many animals show an ecological distribution which correlates with two edaphic formations. This is most noticeably true in case of snails and certain insects, particularly grasshoppers. Earthworms are the most interesting because of their effect on soil conditions. They occur mostly in mesophytic conditions, and show a decided preference for the neutral to slightly alkaline Houston and alluvial soils. Here they are larger and more numerous than in the moist sour Susquehanna clay or Norfolk sandy soils. Their presence probably has some effect on these mesophytic associations.

Grazing affects all the formation types, and often obscures the evidence of dominant forms. Overgrazing is usual during certain seasons. The species of *Andropogon*, the dominant native grasses, are suppressed almost completely in such areas. *Capriola dactylon* takes almost complete possession of the better soils. *Axonopus compressus* forms an almost continuous mat over the less productive fine sandy loams and moist places in the Susquehanna clay. The *Aristata* grasses are the most frequent on sterile xerophytic habitats. In sands they are accompanied by several *Panicum* grasses, and on limy clays by *Buchloe dactyloides* westward of the Willis area. Grazing favors pine forest perpetuation, but prairie continuance is not favored by grazing. This latter has been emphasized by BRAY (1).

Classification and description of plant communities

The most natural and consistent division of this climatic region is on the basis of soil maturity, the edaphic formation type of the leached potentially acid soils, the edaphic formation type of the unleached alkaline soils, and the edaphic formation complex of the mixed soils, circumneutral in their reactions.

A. EDAPHIC FORMATION TYPE OF UPLAND LEACHED (OLD) SOILS

1. Xerophytic formation, type of upland ridges

Quercus minor and *Q. marylandica* are the most constantly occurring tree forms in this formation type, and generally become subdominant to *Pinus echinata* east of the 96th meridian. These dominant species do not showa high coefficient of fidelityto this formation.

(*a*) Association type of loose, constantly aerated soils.—The following plants show considerable fidelity to this type. Trees and shrubs: *Pinus echinata*, *Quercus brevifolia*, *Q. marylandica*, *Castanea pumila*, *Prunus mitis*, *Schmaltzia aromatica*, *Ascyrum hypericoides*, *Ceanothus americanus*, *Vitis Linescomii*, and *Croton argyranthemus*. Grasses: *Andropogon scoparius*, *Stipa avenacea*, *Aristida intermedia*, and *Cenchrus tribuloides*. Herbaceous perennials: *Morongia angustata*, *Astragalus obcordatus*, *Lespedeza hirta*, *Cnidoscolus texanus*, *Asclepias tuberosa*, *A. amplexicaulis*, *Lithospermum Gmelini*, *Physalis intermedia*, *Laciniaria elegans*, *Berlandiera dealbata*, *Froelichia campestris*, and *Chrysopsis graminifolia*. Annuals: *Richardia scabra*, *Linaria texana*, *Heterotheca subaxillaris*, and *Isopappus divaricatus*.

(*b*) Association type of poorly aerated soils with plastic clay subsoils.—The following plants reach their greatest frequency in this association, although none of them are restricted to it. Trees and shrubs: *Quercus minor*, *Hicoria glabra*, *Diospyros virginiana;* also *Panicum anceps*, *Aristida purpurascens*, *Lespedeza procumbens*, *Meibomia obtusa*, *Stylosanthes biflora*, *Plantago aristata*, and *Chaetopappa asteriodes*. This association is poorly defined and limited in its occurrence, appearing mainly on outcrops of Susquehanna clay. It is a very dry soil in drought periods due to puddling and evaporation, and is usually an acid soil.

2. The semixerophytic formation of ordinary uplands

This type embraces all the fine sandy loams of the Norfolk, Tifton, Ruston, Orangeburg, and Susquehanna soil series, occurring upon the ordinary uplands east of the 96th meridian. It constitutes about 90 per cent of the forest in this region, and is the present forest

climax of this character of soil. No attempt will be made to split this formation into its poorly defined associations. Pines are the dominant species, except in a few limited areas where the better phases of the Orangeburg and Susquehanna approach the Greenville series in a higher content of bases. *Pinus echinata* is more xerophytic and more northern in its frequency distribution than *P. taeda*. Where subsoils are rich in ferruginous compounds and usually high in acidity, the pine that is most likely to occur is *P. echinata*. Trees which occur occasionally are *Quercus digitata*, *Liquidambar styraciflua*, and *Ulmus alata*. *Callicarpa americana* is dominant throughout the forests of this type. *Ilex vomitoria* is the most frequent shrub in sunny situations in the southern part of this area. Other plants that reach their greatest frequency in this formation type are *Andropogon virginicus*, *Festuca octoflora*, *Panicum lanuginosum*, *Tridens flava*, *Trifolium carolinianum*, *Cerastium viscosum*, *Syntherisma sanguinale*, *Mollugo verticillata*, *Amaranthus spinosus*, *Hedeoma hispida*, *Rumex hastatulus*, *Salvia lyrata*, *Plantago virginica*, *Viola septemloba*, *Vernonia texana*, *Eupatorium compositifolium*, *Rudbeckia hirta*, and *Diodia teres*.

3. Mesophytic formation type of lower slopes and flatwoods

This association complex is protected from high transpiration by topographical and vegetational features. The soil water is usually abundant, and the water table is at a shallow depth. *Pinus taeda* is the dominant tree, being at least ten times as frequent as *P. echinata*. *Liquidambar styraciflua* is next in importance in its dominance. *Quercus alba* is usually present but is dominant in very limited situations. *Magnolia foetida* and *Ilex opaca* occur mainly in the proximity of stream courses. *Myrica cerifera* is the dominant shrub, especially where the soil structure is loose and the water table shallow; *Callicarpa* prefers well drained soils. Other shrubs are *Viburnum molle*, *Rubus argutus*, and *Gelsemium sempervirens*. Herbaceous plants are *Uniola longifolia*, *Axonopus compressus*, *Eupatorium coelestinum*, *E. semiserotinum*, *E. rotundifolium*, *Aster vimineus*, *Solidago celtifolia*, *Elephantopus carolinianus*, *Lespedeza stricta*, and *Serinea oppositifolia*.

4. Hydro-mesophytic type of wet sour soils

This formation includes the associations of wet sour habitats such as seepy slopes and sandy swamps, and does not include the ordinary lowlands and stream margins. Such soils are usually neutral or slightly alkaline in their reaction, and have been included in the type of the alluvial mixed soils. Some plants in the following list were not observed in the Willis area but occurred in sandy swamps nearby. Trees and shrubs: *Magnolia virginiana*, *Nyssa sylvatica*, *Acer rubrum*, *Myrica cerifera*, *Rhus vernix*, *Vaccinium corymbosum*, *V. stamineum*, *V. virgatum* var. *tenellum*, *Smilax laurifolia*, *Azalea viscosa*, and *Ascyrum hypericoides*. Herbaceous plants: *Xyris difformis*, *Drosera brevifolia*, *Sarracenia flava*, *Eriocaulon decangulare*, *Juncus validus*, *J. scirpoides*, *Panicum rostratum*, *Ibidium cernuum*, *Ptilimnium capillaceum*, *Ludwigia alternifolia*, *L. glandulosa*, *Lobelia puberula*, *Eupatorium rotundifolium*, *Pluchea foetida*, *Osmunda regalis*, *O. cinnamomea*, and *Sphagnum* sp.

B. EDAPHIC FORMATION TYPE OF UPLAND UNLEACHED SOILS

1. Xerophytic formation type of shallow, eroded, limy soils

This is the driest, best aerated, most calcareous, least organic and least productive soil of the Houston series. The most characteristic ecological forms are the herbaceous perennials *Andropogon saccharoides* var. *laguroides*, *Melilotus alba*, *Petalostemon multiflorus*, *P. purpureus*, *Astragalus crassicarpus*, *Verbena bipinnatifida*, *Houstonia angustifolia*, *Grindelia lanceolata*, and *Rudbeckia missouriensis*. These show a high constancy and fidelity to this habitat. Other species showing a preference for this habitat are *Ceanothus ovatus*, *Aristida oligantha*, and *Heliotropium tenellum*. One of the most gorgeous wild flowers in America, the Texas bluebell (*Eustoma Russellianum*), occurs in profusion on this and the following type. Attempts to grow it on any but a limy soil have been unsuccessful.

2. Semixerophytic formation type of ordinary uplands

This association complex is typically prairie, and occupies the most extensive area of this soil type. The soil is characteristically

black, deep, and well supplied with plant nutrients. The pioneer dominant is *Sorghum halepense*. The climax dominants are arranged according to their rank. *Andropogon saccharoides* var. *laguroides*, *Sporobolus Drummondii*, *Stipa leucotrica*, and *Andropogon furcatus*. Trees and shrubs occur sparingly in certain favorable situations: *Ulmus crassifolia*, *Celtis mississippiensis*, *Toxylon pomiferum*, *Gleditsia triacanthos*, *Quercus texana*, *Prosopis glandulosa*, *Svida asperifolia*, and *Crataegus viridis*. Perennials: *Acuan illinoensis*, *A. leptoloba*, *Hartmannia speciosa* (white form), *Vicia texana*, *Physalis longifolia*, *Silphium asperrimum*, *Helianthus Maximilianii*, and *Solidago altissima*. Annuals: *Panicum capillare*, *P. fasiculatum*, *Amphiachyris dracunculoides*, *Bifora americana*, *Plantago rhodosperma*, *Draba cuneifolia*, *Monarda dispersa*, *Croton monanthogynus*, *Centaurea americana*, and *Lindheimera texana*.

3. Mesophytic formation type of lower slopes and more level soils

This type has a lower alkalinity and transpiration rate than the preceding. Trees tend to dominate this association. All of the trees mentioned in the semixerophytic type occur here. Other trees and shrubs are *Fraxinus americana*, *Morus rubra*, *Ulmus americana*, *Cercis canadensis*, *Ilex decidua*, *Smilax Bona-nox*. Grasses: *Andropogon furcatus*, *A. glomeratus*, *Tripsacum dactyloides*, *Sorghum nutans*. Herbaceous perennials: *Hartmannia speciosa* (pink form), *Dolicholus minimus*, *Neptunia lutea*, *Mesadenia tuberosa*, *Aster salicifolius*, and *Carduus austrinus*. Annuals: *Dichrophyllum bicolor*, *Hordeum pusillum*, *Leptochloa mucronata*, *Chaetochloa lutescens*, *Trepocarpus aethusae*, *Chamaesyce nutans*, *Astragalus Nuttalliana*, *A. reflexus*, *Dracopsis amplexicaulis*, *Senecio lobatus*, and *Sitilias multicaulis*.

4. Hydro-mesophytic and hydrophytic formation-complex of alkaline depressions and lowlands

Trees and shrubs: *Fraxinus lanceolata*, *Ulmus americana*, *Hicoria aquatica*, *H. pecan*, *Populus deltoides*, *Planera aquatica*, *Gleditsia aquatica*, *Ampelopsis cordata*, and *Vitis cinerea*. Herbaceous plants: *Uniola latifolia*, *Elymus virginicus*, *Lythrum ovalifolium*, *Meibomia paniculata*, *Koellia flexuosa*, *Iva ciliata*, *Ambrosia trifida*,

Helianthus Maximilianii, *Helianthus grosseserratus*, and *Aster exilis*. The aquatics are *Chara*, *Potamogeton*, and *Typha*.

C. EDAPHIC FORMATION TYPE OF MIXED SOILS, USUALLY NEUTRAL IN REACTION

1. Transitional zone between leached and unleached upland soils

This is the tension zone between prairie and forest. These soils are much the same as those of the Lufkin series. They normally possess silty fine sandy loam surface soil and a plastic clay subsoil. This type ranges from mesophytic to xerophytic conditions, dependent upon the topography and drainage. *Quercus minor* is the dominant tree. *Crataegus spathulata*, *Celtis mississippiensis*, *Prunus americana*, and *Pinus taeda* are the dominant species. Mesophytic situations are particularly favorable to the development of *Pinus taeda*. *P. echinata* is infrequent in this zone. Other plants showing a preference for this zone are *Chamaecrista fasciculata*, *Sabbatia campestris*, *Panicum virgatum*, *Laciniaria pycnostachya*, *Gaura longiflora*, *Symphoricarpus symphoricarpus*, *Ruellia ciliosa*, *Ptilimnium Nuttallii*, and *Erigeron tenuis*.

2. Associational complex of alluvial soils

The soils of this association are usually slightly alkaline and well supplied with nutrients. This perhaps explains why the associations of the Houston soils are better represented here than on the leached upland soils. This formation complex also contains a large number of plants of the upland sands. It is here that the mesophytic types of the two edaphic formations merge into one complex. The hardwoods are the dominant trees. Lianas reach their best expression in this habitat.

Plant communities of special indicator significance

1. Disjunct prairies of Montgomery County, Alabama.—This is a natural upland prairie on Houston black clay. It presents much the same ecological and floristic aspect as is observed on this soil type in Texas. Estimates on an undisturbed area indicate that 75 per cent of the plant cover is occupied by species common to the

Texas prairies, as *Andropogon saccharoides, Sporobolus Drummondii, Hartmannia speciosa, Monarda dispersa, Croton monanthogynus, Acuan illinoensis, Petalostemon purpureus, Houstonia angustifolia, Heliotropium tenellum,* and *Polytaenia Nuttallii.* Common weeds in disturbed areas are *Rudbeckia amplexicaulis, Iva ciliata, Ambrosia trifida, Aster exilis, Trepocarpus aethusae, Sida cordifolia,* and *Sorghum halepense.* These species are normally absent from sandy areas nearby. This prairie formation on upland calcareous soils in the midst of the eastern climatic forest is an indication that the vegetation expresses the youth or maturity of the soil.

2. DISJUNCT PINE FOREST OF BASTROP COUNTY, TEXAS.—This area is 80 miles west of other pine forest and in the tall grass prairie region of the state. *Pinus taeda* forms a typical pine forest on an area of 40 square miles of Norfolk fine sand and a small amount of loose Susquehanna gravelly loam. To recount the species common to this and to the pine forest of the Willis area would make too long a list. Mention may be made of some of the more interesting members: *Svida florida, Vitis Lenscomii, Myrica cerifera, Drosera brevifolia, Laciniaria elegans, Eupatorium coelestinum, Hypoxis hirsuta, Ilex vomitoria, Cracca virginiana,* and *Isopappus divaricatus.* The most notable for its absence is *Pinus echinata.* The rather xerophytic habitat suggests that it rather than *P. taeda* would be expected here. Two possible causes for its absence are suggested: (1) that *P. echinata* extended its range southward in Texas since this area was cut off from the eastern forest; (2) that *P. echinata* is less tolerant of high temperatures than *P. taeda.* This area occurs in the latitude of the southern limit of *P. echinata.* This disjunct area is an example of a pine forest being retained west of its climatic range by edaphic conditions.

3. CALCAREOUS SANDS OF COASTAL STRAND OF EASTERN TEXAS.—Many species common to leached sour sands are lacking in these soils, and many species common to calcareous Houston clay occur on these calcareous sands, although they are rare on leached sands contiguous to the prairies. The following plants, frequent upon the calcareous soils of the prairie, occur here: *Acuan illinoensis, Dichrophyllum bicolor, Chamaesyce nutans, Croton monanthogynus, Helianthus Maximilianii, Gaura parviflora, Iva ciliata, Ambrosia trifida,*

Grindelia lanceolata, *Houstonia angustifolia*, *Dolicholus minimus*, *Scutellaria Drummondi*, *Gleditsia triacanthos*, and an occasional *Quercus virginiana*. The preference of these species for unleached soils seems to be independent of soil texture and aeration. A number of other plants show a preference for sand that is not correlated with a leached condition, as *Heterotheca subaxillaris*, *Daucus pusillus*, *Monarda punctata*, and *Erythrina herbacea*. Some show a preference for unleached sand, as *Gaillardia pulchella* and *Argemone alba*.

4. BIG THICKET OF TEXAS.—A mesophytic climax forest type much like the Tennessee forest type joins the *Pinus taeda* forest of the Willis area on the east, and is known locally as the Big Thicket because of the luxuriance of its undergrowth. Its area covers about 1000 square miles, and its shape is that of a delta, with the apex inland near Livingston and its base extending almost to the coastal prairies to the south. On the eastern boundary it is joined by the *P. palustris* forest of the coastal flatwoods. Its topography is much the same as that of the adjoining region, except that it is traversed by the Trinity River and its floodplains, and is more broken by stream courses making it less subject to fires. The soils are fine sandy loams known locally as hammock lands, and are circumneutral in their reaction. The dominant species are *Quercus alba*, *Q. phellos*, *Fagus americana*, *Acer saccharum*, *Magnolia foetida*, and *P. taeda*. It seems improbable that this climax vegetation and the accompanying circumneutral condition of the soils would become general throughout eastern Texas on leached uplands as a result of increased mesophytism. This formation is really south of its climatic range, and owes its preservation to local influences that protect it against the ordinary extremes of climate and to a soil which is probably younger and more fertile than other sandy loam soils of the adjacent areas.

5. PLANT COMMUNITIES AS INDICATORS OF SOIL RELATIONSHIPS.—A good idea of the edaphic formation relationships and distinctions was obtained by determining the number of plants that were frequent on one soil and infrequent on an associated soil. The following is a summary of the species having preference for certain soil types, determined by their percentage of frequency. Of the 178 species frequent on Norfolk sand, 118 seldom occur on Houston black clay,

20 seldom occur on red clay, and 11 seldom occur on Norfolk fine sandy loam. There are 161 species frequent on Houston black clay. Of this number, 101 seldom occur on sand. On red clay there are 163 frequent species. Of this number, 103 seldom occur on Houston black clay and 5 seldom occur on Norfolk sand or Norfolk fine sandy loam. These figures show the close correlation between the vegetation in soil types of low basicity, and they indicate that the plants of Norfolk sand and Norfolk fine sandy loam and Susquehanna clay should be included in one plant formation. Except for a small number of indicator species, differences in vegetation of these soils are largely differences of valence of species.

TABLE VI

INDICATOR SIGNIFICANCE OF ANNUALS IN NORFOLK AND HOUSTON SOILS

HABITAT	ALL ANNUALS	WINTER ANNUALS	SUMMER ANNUALS
Plants showing a strong preference for			
Sandy soils	50	32	28
Black clay soils	55	31	24
Plants common to both	72	40	32
Total	177	103	84

6. ANNUALS AS INDICATORS.—Annuals are important indicators, especially in cultivated ground, as shown in table VI. Under the winter annuals are included all those species that occur from seeds germinated by fall rains, and that mature their seed before the middle of June. Vegetation during this period seldom suffers from lack of moisture. That the percentage of difference for the winter annuals of the Houston black clay and of the sandy soil types should be little greater than that of summer annuals is an argument against considering water as the dominant factor in accounting for the differences in the vegetation of these soils. The most striking indicators of sufficient calcium carbonate in a soil are observed in two summer annuals, *Dichrophyllum bicolor* and *Amphiachyris dracunculoides*. These are so frequent in late summer and autumn that non-acid soils may be excellently mapped by their distribution.

Succession

Succession in this region is associated with the slowly operating processes, leaching and leveling. The associations are so nearly stable that any prediction of change must be largely speculative. The two most extensive and best developed associations are the pine forest and the prairie, but these are apparently the least stable ones, and there is evidence in places of their losing ground. These also are the most aggressive pioneering associations. Their quick invasion following catastrophes is sufficient explanation of their occupation of many situations.

The recent more rapid invasion of prairies by members of other associations is a result of overgrazing which permits the entrance of certain woody species, but the slower and more permanent invasion occurs under natural conditions as a result of leaching, change of texture, and stabilization of the soil. These conditions bring in the xerophytic low oak associations in dry situations, and the tall broad-leaf hardwoods and *Pinus taeda* in the less dry situations. Thus it would seem that the prairie areas of eastern Texas are destined to become deciduous forests of scrub oak or tall oaks, depending upon the degree of mesophytism. Such a state is remote under natural conditions, due to the effects of surface erosion which prevent these soils from maturing.

The ultimate climax type of the upland leached soils is more difficult to predict than that of the prairie. Pines are the pioneering trees, and remain as the dominant type where the soils are sufficiently sterile not to produce a floor covering which will shade out their seedlings. Pines, once established on sandy soil, tend to hold their dominance by their superior height and by their deep and extensive root competition. If conditions should become sufficiently fertile or mesophytic, pines would be crowded out by shading. At present the tall oak dominates in east Texas only on the slightly limy, fertile soils of the Greenville series and in certain better phases of the Orangeburg series. Pines dominate all leached soils, mainly the upland sands, westward to the limit of their climatic range. If the pine forest should become much more xerophytic, the post oak association would be the climax type. If conditions should become mesophytic, it is possible that the white oak-magnolia-beech-maple

association would come into dominance. The latter association is only incompletely expressed in the Willis area on hammock land near water courses, where transpiration is low and the summer temperature is reduced. It reaches a rather complete expression in the Big Thicket east of the Willis area. The pine forest under present conditions shows little tendency to develop a mesophytism that will enable the invasion of other forest types. Pine seedlings develop readily on the forest floor. Cut-over woodland returns to pine. This may be obscured at first by an open growth of young hardwoods, but in the course of about fifteen years pines have gained an ascendency over the less rapidly growing hardwoods. The factors that operate to continue this dominance are: (1) the poverty of the soil which prevents shading out of seedlings; (2) the lack of a well developed layer of leaf mold, due to its destruction by microorganisms and frequently recurring ground fires; and (3) the structure of the soil that favors leaching and percolation of solutes favorable to deep feeding pine roots.

The low oak association, *Quercus minor* and *Q. marylandica*, is the climax type on all leached soils west of the pine forest and east of the shortgrass plains to the northwest and of the desert scrub to the southwest. This association is well stabilized and is gaining ground slowly as the Houston soils become more leached. This gain upon the prairie is not due to increased mesophytism but to leaching. The herbaceous vegetation of this association is more scanty and apparently as xerophytic as the associated prairies. This is the most stable and strikingly edaphic formation type of this region.

The mesophytic hardwoods dominate the stream valleys and certain other situations where the soils are somewhat fertile. Certain species show a tendency to extend their range into the pine forest, where conditions have become more mesophytic, and in these situations when pines are removed the hardwoods apparently remain dominant. This permanent supplanting of pines is infrequent in the upland soils of the Willis area. In general it can be said that the tall hardwood association is not only holding its own but probably gaining ground.

The retrogressive factors which influence succession are as follows: (1) fire, which aids in the dominance of pine forest on leached

soils and prairie grasses on unleached soils; (2) grazing, which favors pine and xerophytic oaks; (3) lumbering of pine, which encourages the dominance of hardwoods; (4) the reversion of old fields, once cultivated, to pine forest or prairie depending on the character of the soil.

Summary

This paper is an attempt to present data on the relative value of different factors operating to determine edaphic formations on certain soil types. In order to define the plant communities it has been necessary to divide them on the basis of leached or unleached conditions of the soil, on the influence of water and topography, and on the nature of soil texture and aeration. The valence of abundance has been estimated for most of the members of each community, and the probable steps in succession have been considered. The climax types of vegetation have been named, and some of the conditions producing them have been presented.

1. Soil texture and aeration as factors in plant distribution are expressed (a) in the vegetation and in the decided difference in the valence of frequency of each species with changes in soil texture; (b) by the occurrence of a number of species in dry, well aerated sands that are infrequent in dry poorly aerated leached clays; (c) by the presence of a number of silicicoles common to alkaline and leached sands and infrequent upon clay soils; (d) by difficulty of root development in the subsoil of prairies of this area; and (e) by the intolerance of certain plants to destruction of lateral roots by soil cracking. Much of the difference in the structure of the vegetation of the respective soil types of this area is due to soil texture and aeration.

2. Water apparently is not the major controlling factor in determining the difference in the structure of edaphic formations under consideration, although it is an important factor in the xerophytic, semixerophytic, mesophytic, and hydrophytic divisions of edaphic formations. Evidence that water may be of minor importance is shown by the societies of winter annuals and weeds on cultivated soils which are little affected by the water differences. The conditions of leached upland forested ridges apparently are more xerophytic than those of less exposed prairies. The vegetation of the

former shows a higher fatality rate than that of the prairie. Neither transpiration nor soil water explains adequately the difference in xerophytic forest and prairie of eastern Texas.

3. The maturity of the soil as indicated by its chemical nature is a very important factor in plant distribution. Apparently it is the major factor in determining the edaphic formations of this climatic region, as shown by (a) characteristic weed societies for cultivated soils of unlike chemical nature yet similar in being well aerated and supplied with moisture; (b) occurrence of distinct winter annual societies on leached and unleached soils; (c) the absence of calcicoles upon leached plastic clay although frequent upon calcareous plastic clay; (d) the frequency of most silicicoles upon leached plastic clay and the infrequency of these on calcareous plastic clay; (e) the difference in the associations on calcareous and leached sand; (f) the lack of similarity in the hydro-mesophytic associations of acid swamps and alkaline swamps; (g) the presence of species common to alluvial and upland unleached soils but infrequent or absent on upland leached soils; (h) the qualitative and quantitative differences in microorganisms of soils of contrasting chemical characteristics.

4. In considering biotic factors it was found that (a) where bacteria are abundant, producing an accumulation of mild humus, soils become dark and productive, and where fungi are abundant decomposition becomes more complete and soils are gray or yellow and unproductive; and (b) grazing favors pine forest perpetuation and is unfavorable to prairie continuance.

5. Fire is very favorable to the perpetuation of prairie by the destruction of shrubs, small trees, and seedlings. Intermittent fires favor the dominance of pine forest by the destruction of forest litter, thus checking mesophytism and soil improvement.

6. The plant associations considered are the most definite where soil conditions offer the greatest number of distinctive chemical and physical characteristics, as when a limy Houston clay is associated with leached Norfolk sand. Of the 178 species frequent on Norfolk sand, 118 seldom occur on Houston clay; and of the 161 species frequent on Houston clay, 101 are infrequent on sand.

7. The plant associations are less distinct where they occupy soils which differ mainly in one of these groups of factors; yet this differ-

ence may be very striking where soils differ greatly in chemical nature, as in Susquehanna clay and Houston clay. Of the 163 species frequent on sour Susquehanna clay, 103 seldom occur on calcareous Houston clay. This difference is less marked where the factor differences vary widely physically, as in Norfolk sand and Susquehanna clay, than where they vary widely chemically. Of the 178 species frequent on Norfolk sand, 20 seldom occur on Susquehanna clay.

8. When two soils of only slight difference are associated, as in Tifton and Norfolk or Ruston and Orangeburg, it follows that the vegetational differences will be slight. These differences will be mainly in frequency of the plants.

9. The majority of plants show preference for certain combinations of edaphic factors. Few are ubiquitous to all soils in anything like an equal frequency.

10. Edaphic factors exert their minimum influence in mesophytic conditions but are quite marked in xerophytic and hydrophytic conditions. Mesophytic associations in the Willis area are very limited in their occurrence. The uplands are generally occupied by semixerophytic and xerophytic associations.

11. That edaphic factors may upset climatic expectations is shown in the occurrence of disjunct prairies in the forest region of central Alabama on Houston clay, and of disjunct pine forest in the prairie region of central Texas on Norfolk fine sand.

12. The six dominant associations represent in general six types in physiognomy; namely, the prairie, the low upland oaks, the tall upland oaks, the pine forest, the lowland hardwoods, and the mesophytic climax forest. (a) The prairies in this area are characterized by a factor complex as follows: a good supply of mineral nutrients (especially lime), a maintenance of grass competition for soil oxygen and moisture, a tolerance for the destruction of lateral roots by soil cracking, recurring fires, a soil texture that is unfavorable to deep or extensive root systems, and xerophytic conditions in late summer and autumn. The dominant grasses are *Andropogon saccharoides* var. *laguroides*, *A. furcatus*, *Sporobolus Drummondii*, and *Stipa leucotricha*. (b) The low oak association is characterized by sparse floor covering, which limits competition for soil oxygen and favors the penetration of oxygen to the subsoil, a lower content of clay in the

surface soil, lack of excess lime, and climatic xerophytic conditions. Dominant plants are *Quercus minor*, *Q. marylandica*, and *Hicoria glabra*. (c) The tall upland oak association is dependent on a medium supply of water and mineral nutrients. Dominant species are *Q. digitata* and *Q. velutina*. (d) The pine association is dependent on little competition, adequate water, illumination for seedling development, and a soil structure favorable to their root development. (e) The lowland hardwood association is characterized by an abundance of mineral nutrients and soil water. The dominant types are *Fraxinus americana*, *F. viridis*, *Ulmus americana*, *Quercus nigra*, *Q. phellos*, *Populus deltoides*, *Hicoria pecan*, and *Platanus occidentalis*. Lianas are frequent. (f) The mesophytic climax forest is characterized by abundant supply of water, low evaporation, low summer temperature, and infrequent occurrence of fires. This association is very limited in this area, but reaches a full expression just east of it in the Big Thicket, where the dominant plants are *Quercus alba*, *Liquidambar Styraciflua*, *Nyssa sylvatica*, *Ilex opaca*, *Magnolia foetida*, *M. virginiana*, *Fagus americana*, *Acer saccharum*, and *Pinus taeda*.

13. The six dominant associations are all nearly stabilized, but there is some evidence that the prairies are losing ground slowly under the influence of leaching, and more rapidly under the influence of grazing and cessation of prairie fires, and that the pine forest is losing ground slowly under the influence of mesophytism.

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EFFECTS OF X-RAYS UPON GROWTH, DEVELOPMENT, AND OXIDIZING ENZYMES OF HELIANTHUS ANNUUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 360

EDNA LOUISE JOHNSON

(WITH ELEVEN FIGURES)

Introduction

The nature of the effects of X-rays upon seeds and seedlings is still in need of investigation. This paper deals particularly with the physiological effects resulting when various doses of X-rays are applied to seeds and young seedlings of *Helianthus annuus*. Few investigations up to this time have included observations continued until the end of the life cycle of the irradiated plants. When plants are subjected to changed conditions of any sort, there is a necessity for continued observation until maturity, before definite conclusions can be stated. In these studies, a sufficient number of plants were allowed to grow to maturity, so that it was possible to observe not only their immediate response to irradiation, but also any effects appearing during later development.

Results of various experimenters often have not agreed because the observations were made on different species of plants, or were carried out on seeds with varying water content, or employed various units of dosage. Some (**25, 26**) have observed acceleration of germination with weak irradiation, while a recent investigator (**4**) found none. Several (**1, 24, 33**) have reported acceleration of growth due to weak irradiation. Practically all have agreed that soaked or germinated seeds are more sensitive than dry resting ones. Experiments by the writer show that this is true also for seeds of *Helianthus*.

Very little has been done to show the relation of X-rays to abnormalities of form and to changes in internal structure. These subjects, together with a study of the physiological effects of X-rays upon respiration and the oxidizing enzymes, will be considered in this paper.

Materials and methods

The seeds used for the experiments were those of the so-called Russian sunflower, *Helianthus annuus*, from which the ovary wall was removed before irradiation. Air-dried seeds were found to contain 3.41 per cent water. Since others (**1, 16, 21, 26**) have found that the sensitivity to X-rays is affected by the water content of the seeds, this will be recorded in all cases.

The unit of dosage employed in the experiments to be described is the "human erythema" dose of Ivy (**19**), and is designated by E. The "set-up" for one erythema dose is 60 K.V.M. (maximum kilovoltage), 5 milliamperes current, no filter, 30 cm. focal distance, 5 cm. portal of entry for 5.5 minutes.

For irradiation[1] the seeds were placed in open Petri dishes so arranged that the rays came vertically from above. Care was taken to treat the control seeds exactly as the irradiated ones, except for lack of exposure to the rays. Within a short time after irradiation, both irradiated seeds and controls were planted in 8-inch pots and all were placed under similar environmental conditions in the University of Chicago greenhouse. In most cases, ten seeds were used to a pot. After a period of from ten days to two weeks the plants were thinned, and only two were allowed to grow to maturity in a pot. Any exceptions to this procedure will be noted.

Since many (**15, 16, 21, 26**) have reported that plants differ in their sensitivity to X-rays according to the species, it was first necessary to determine the lightest amount of dosage which would affect the seed of *Helianthus annuus* in any manner, and also the smallest dose which would noticeably inhibit growth. The first was found to be between 1 E. and 5 E., while the latter was 10 E. Those seeds receiving heavy irradiation (20 E.) produced seedlings which died after developing to the point where a portion of the cotyledons appeared above the soil.

I. Action in causing fasciation

A very constant effect of X-rays on seeds and seedlings of *Helianthus annuus* is the production of fasciation in stems, leaves, and

[1] The X-ray equipment used was lent to Dr. A. C. Ivy, formerly of the physiology department of the University of Chicago, by the Standard X-ray Company of Chicago, and by Dr. Ivy placed at the disposal of the writer.

flowers. In the stems this is expressed by the flattened strap-shaped or ribbon-like expansion of the main stem. At the base the stem is generally cylindrical, but the apex may be combed or diffusely branched (fig. 1). The stems after becoming flattened usually show bifurcations or splittings somewhere along their length. The resulting branches sometimes remain unfasciated, but in many cases they themselves become divided again (fig. 2). Very often an indentation or groove, which becomes wider and wider as the stem flattens, runs up a portion of the stem.

FIG. 1.—Left, control; right, tip of fasciated plant (48 days old) given X-ray dose of 5 E. when 4 cm. tall; viewed from above.

Fasciation is shown in leaves by changes in their number and position, and by changes in size and shape. Commonly the number of leaves is increased and phyllotaxy is distorted. Some of the leaves are normal, some are minutely notched at the apex, some are forked for one-third their length, and others are deeply forked. In extreme cases an original single leaf is split into two independent leaves, attached at the same point on the main stem (fig. 3). Many of the leaves in the growing tip show incurling and ruffling of the margins.

Fasciations of the flower were present in various forms; fusion sometimes took place in the involucral region, giving the appearance of twin heads (fig. 4). In other cases the flower stalk forked a short distance below the insertion of the flowers and two distinct heads were observed.

Investigations of others (**13, 18, 22, 23, 31, 39, 42**) suggest that fasciation may be due to: (1) suitable conditions for rapid development; or (2) sudden arrest of the activity of the growing point due to insect or fungal attacks; or (3) mechanical injury to the growing point.

There is no doubt that fasciation is induced by the action of X-rays on seeds and seedlings of *Helianthus*. This has been proved by the writer in repeated experiments. Seeds containing 57.3 per

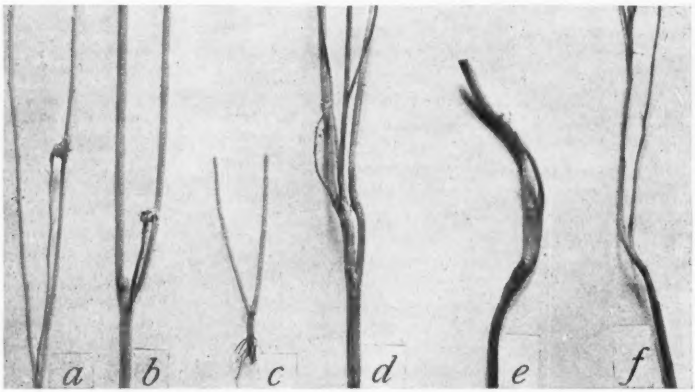

FIG. 2.—Types of fasciated stems from: *a*, plant whose seed had received dose of 7 E.; *b*, seed which had received dose of 10 E.; *c*, seed which had received 10 E.; *d*, seedling which had received 5 E.; *e*, seedling which had received 5 E.; *f*, seed which had received 8 E.

cent moisture were put into germinators for about 17 hours and then placed in short cylindrical packets of moist sphagnum. After three or four days' growth, the young seedlings were exposed to a dose of 5 E. The focal distance (30 cm.) was measured from the growing tip of the stem. The packets were then placed in soil without disturbance to the young seedlings. During early stages of growth of the seedlings and before fasciation appeared, the effects of irradiation were similar to those seen in young plants whose seeds had been irradiated. Aside from checking of growth, the first striking effect was noted in the abnormally shaped young foliage leaves with their pitted or pebbly appearance. The effects were seen only in

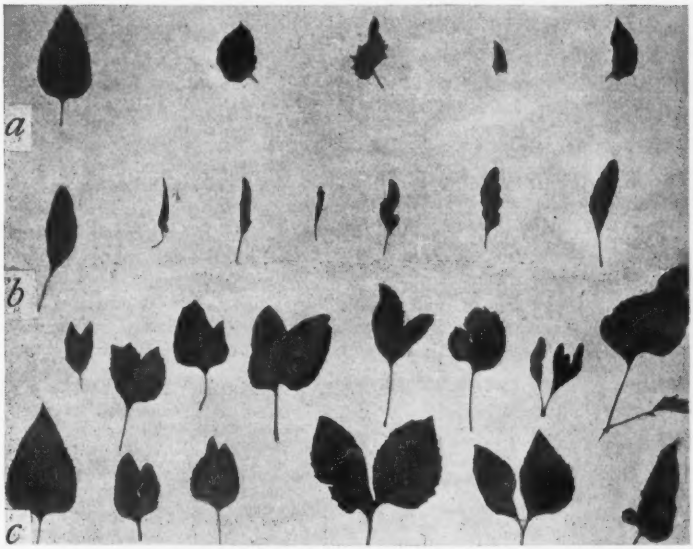

FIG. 3.—First leaf of each row, control; *a*, typically deformed leaves from upper regions of irradiated plants; *b*, abnormally ligulate leaves from lowest nodes; *c*, incipient fasciation shown by leaves.

FIG. 4.—Fasciated flower head produced on plant irradiated in young seedling stage.

foliage leaves, never in cotyledons. Table I shows the course of development of the young plants. The dates given for time of blossoming are from appearance of first flower to appearance of last. Height is taken from base of stem to highest growing point.

In other experiments by the writer, clear cases of fasciation were induced also in tomato and cosmos seedlings by means of X-rays. Various abnormalities appeared, as flattened stems, dichotomously branched main stems and lateral branches, and altered phyllotaxy.

TABLE I

FASCIATION, DECREASED TOTAL GROWTH, AND EARLY BLOSSOMING SHOWN AS RESULTS FROM IRRADIATING YOUNG SEEDLINGS WITH A DOSAGE OF 5 E.

SERIES NO.	HEIGHT AT TIME OF IRRADIATION (CM.)	HEIGHT AT TIME OF BLOSSOMING	TOTAL DAYS OF GROWTH BEFORE BLOSSOMING	WEIGHT AT MATURITY (GM.)		EVIDENCE OF FASCIATION
				Green	Air-dry	
KS.3*a*...	4	198	119	108.9	.....	Dichotomous branching 17 cm. from base of stem (greatly flattened)
KS.3*b*....	4	135	101–113	72.5	.....	Dichotomous branching 50 cm. from base of stem; 6 branches formed, all blossomed
KS.3*c*....	4	124	101–112	.....	21.5	First dichotomous branching at height of 77 cm.; further splitting of stem until 6 tips evident; 6 blossoms, one double
KS.3*d*...	Only arch of hypocotyl showing	152	107	79.2	.....	No abnormalities
KS.3*e*....	Only arch of hypocotyl showing	138	104	.....	17.1	Large double blossom due to splitting in involucral region (fig. 4)
Control...	5	144	112	.....	26.4	Normal one-stemmed plants with single blossom
Control...	Only arch of hypocotyl showing	169	101	94.8	.....	Normal
Control...	Arch of hypocotyl showing	171	127	.....	.....	Normal

In the experiments with *Helianthus* here described, fasciation was induced in 80 per cent of the cases by irradiating seedlings with one dose of 5 E. Experiments described later will record fasciations induced when slightly heavier doses were given to soaked seeds.

II. Proportional effect of dosage and growth

1. IRRADIATED SEEDS.—When seeds with a water content of 58.7 per cent were exposed to dosage ranging from 5 to 10 E., the amount of injury produced during the first three weeks of growth was approximately proportional to the dose. This effect proved to be transitory, and by flowering time the growth of the irradiated plants was almost equal to that of the controls.

Fasciation was present in some of the plants of this series. Fasciation of the stem was induced in 75 per cent of the plants whose seeds received a dose of 7 E.; those whose seeds received 8 E.

TABLE II

ABNORMALITIES PRESENT IN SEEDLINGS; SOAKED SEEDS IRRADIATED WITH DOSAGE DESIGNATED

DAYS AFTER IRRADIATION	APPEARANCE AFTER RECEIVING X-RAY DOSAGE OF					
	5 E.	6 E.	7 E.	8 E.	9 E.	10 E.
13.....	Some leaf distortion throughout entire series Dark and light patches of green giving mosaic appearance Growth of tip inhibited					
29.....	Stem diameter shows considerable increase above first node					
				Dichotomous branching	25% plants dead	50% plants dead; fasciation of main stem
43.....	Slight abnormality of leaves	Young leaves still showing decided abnormalities	Marked abnormality in leaves; one plant showing three tips; one, two tips	Abnormal phyllotaxy and leaf development; plant with three tips	Leaves curled; axillary buds developing in one plant	Abnormal phyllotaxy
50.....	Almost normal	Few abnormal leaves at top	75% fasciation	50% fasciation	See above	Fasciation in both stems
74.....	Apparently normal except for peculiar appearance of basal internode	Slight leaf abnormality; peculiar appearance of basal internode	See above	See above	Leaves large but unsymmetrical; abnormal phyllotaxy	See above
96 (time of blossoming).....	Plants and flowers almost normal	No fasciation; almost normal	Best fasciation shown in stem split 62 cm. from base	Best fasciation in stem with secondary and tertiary splitting; three small blossoms on this plant	No fasciation of stems	One plant with split 87 cm. from base of flattened stem; two blossoms present; other plant with split 23 cm. from base; two normal and one stunted blossoming stem

showed fasciation in 50 per cent of the cases. Of the plants whose seeds received 10 E., all of those that grew to maturity showed fasciation. The type of fasciation in these plants was that of dichoto-

mous branching, where the main stem split up into secondary and tertiary branches. The earliest effect noted in the seedlings of ir-

TABLE III

TOTAL HEIGHT OF IRRADIATED SEEDLINGS MEASURED AT WEEKLY INTERVALS

SERIES NO.	HEIGHT IN CM. AT WEEKLY PERIODS									
	1	2	3	4	5	6	7	8	9	10
OS .35	Dose of 5 E.									
1....	2.0	4.4	7.8	18.0	36.0	52.0	66	78	93	105
2....	2.2	4.5	10.2	22.0	44.0	52.0	68	83	98	108
3....	2.0	5.7	12.5	25.5	46.0	57.0	72	84	97	111
4....	2.0	6.0	11.8	25.5	46.0	59.0	72	84	99	112
5....	2.0	4.2	7.8	18.0	32.0	43.0	54	63	75	86
6....	4.0	8.0	15.2	23.0	41.0	56.0	66	74	90	103
7....	2.5	6.0	11.7	20.0	37.0	54.5	68	76	95	111
8....	2.2	4.6	11.4	23.0	41.5	54.5	64	78	95	105
9....	3.0	5.4	9.8	19.0	37 0	50.0	62	78	90	110
10....	3.0	5.2	9.0	18.6	30.0	36.0	40	48	62	70
11....	2.0	3.2	7.0	17.0	38.0	36.0	71	85	99	103
12....	3.0	4.6	7.0	13.0	23.0	32.5	37	40	60	62
Average.	2.5	5.15	10.1	20.2	37.6	48.5	61.7	72.5	95	99
OS .310	Dose of 10 E.									
1....	1.0	Died								
2....	0.5	2.0	4.2	11.0	20.0	49	61	74	90	107
3....	0.3	3.0	4.4	8.0	10.5	15	26	39	58	78
4....	0.1	2.0	7.2	14.5	20.1	39	50	64	82	98
5....	0.1	2.7	4.7	8.0	27.0	38	54	59	66	74
Average.	0.17	2.4	5.1	10.4	19.4	35	48	59	74	89
	Control									
1....	2.0	7.0	15.0	28	49	72	84	91	103	115
2....	3.0	6.8	12.6	24	40	57	65	75	89	92
3....	2.2	6.6	14.0	24	48	68	75	87	95	98
4....	1.0	4.5	17.0	23	40	62	84	92	100	117
5....	3.0	6.6	15.0	31	49	67	77	88	104	115
6....	1.0	4.4	12.5	24	46	67	75	98	105	115
7....	2.0	4.6	10.5	25	41	69	74	81	93	99
Average.	2.0	5.8	13.8	25.6	45	66	76	87	98	107

radiated seeds was the peculiar mosaic appearance of the young foliage leaves as they unfolded. This seemed to be due to the irregularity of the amount of green color in the leaves. As they became

older the distribution of chlorophyll became normal. Abnormalities in shape of leaves from lower nodes tended to disappear as the plants grew older. Table II records the appearance of plants of this series at certain intervals.

2. IRRADIATED SEEDLINGS.—The growth of irradiated seedlings throughout their development appears to be almost inversely proportional to dosage. Seedlings were prepared for irradiation by the

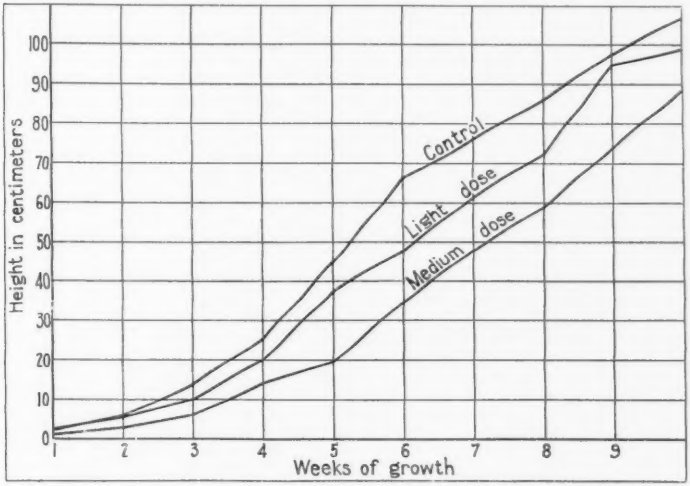

FIG. 5.—Curves showing total growth of control plants and those irradiated in seedling stage with light and medium doses; growth measured at weekly intervals.

method described, and when approximately 2 cm. in height one group was given a dose of 5 E., the second group was given a dose of 10 E., and the third group was used as a control. The experiment was closed at the end of 65 days when all the control plants had bloomed. Very warm days during the growth period (July 8–September 11) probably account for the very short period of blooming. The delay in blossoming seen in seedlings receiving 10 E. is thought to be due to the pronounced stem fasciations present. Repeated divisions of the main stem had resulted in the presence of many branches. At the close of the experiment practically all of these bore buds. The weekly total heights of individual plants of

each group are recorded in table III, together with the average for the groups. Fig. 5 represents the curve of growth based on the average growth for each group. In table IV are given brief descriptions of abnormalities occurring in irradiated seedlings. In those receiving 5 E. fasciation occurred in 66 per cent of the plants, in those receiving 10 E. it occurred in 100 per cent.

TABLE IV

EFFECT OF IRRADIATION UPON YOUNG SEEDLINGS

SERIES NO.	DOSE	CONDITION AFTER 65 DAYS		ABNORMALITIES
		Average height in cm.	Per cent in blossom	
OS .35.....	5 E.	98	66.6	Altered phyllotaxy (three or four leaflets in whorl); leaves spotted and pitted apparently from unequal distribution of chlorophyll; lower leaves ligulate and narrowly lanceolate Types of fasciation: (1) incipient fasciation of leaves similar to that shown in fig. 3; (2) flattened stems; (3) dichotomously branching stems which again divide; (4) double head
OS .310.....	10 E.	67	None	Pronounced fasciation in all plants; stems flattened, grooved, and dichotomously branched; plant shown in fig. 7 (right) divided into branches *A* and *B*. *A* remained unbranched; *B* divided into one short branch (*a*) and one long branch (*b*), *b* split into three branches (one with tip with four buds, other tips had one bud each). Close of experiment at end of 65 days may have prevented further fasciation
Control.....	.....	107	100	None

This experiment indicates that a dose of 10 E. causes very severe injury to plants when they are irradiated in the young seedling stage. Fig. 6 shows appearance of some of these plants sixteen days after irradiation. The cotyledons appear unharmed but the young foliage leaves as they develop show much distortion. After a period of about three weeks there is a partial recovery from injury, and from that time on more normal growth is made. Fasciations were evident twenty-one days after irradiation; these became more pronounced as the plant developed (fig. 7). Here, as in the experiments just preceding, we have evidence that if the plant is not killed by the dose

given, it will partially recover and a normal increment of growth will be made until the plant approaches the height of the controls.

III. Effect upon catalase activity

Since some (**14, 17, 36**) have felt it reasonable to assume that catalase activity can be considered an index to metabolism, experiments were undertaken to ascertain whether inhibition of growth which accompanies heavy irradiation is associated with decreased

FIG. 6.—At right, two 20-day old seedlings irradiated with 10 E. when less than 1 cm. in height (appearance typical for this age); partial recovery from effects of irradiation took place after photograph was taken, and plants eventually reached a height of 67 cm. in 63 days; controls averaged 107 cm. in same period.

catalase activity. The one experiment (**37**) found in the literature which deals with the catalase activity of X-rayed plants, states that the activity of the sweet almond is not affected by irradiation. Those (**28, 37**) experimenting with the catalase of liver have found that irradiation diminishes its activity.

The writer has employed an apparatus modified from that of APPLEMAN (**5**). Throughout the course of the experiments the water bath was kept between 19°–20° C., and all results were calculated to standard conditions of temperature and pressure. The commercial form of 3 per cent Oakland dioxygen, frequently tested by the potassium permanganate method, was used. During the course of

the experiments the plant tissue and dioxygen were kept alkaline by the addition of decinormal NaOH and calcium carbonate. In one group of experiments the plant material used was powder, prepared by drying seedlings in the laboratory for seven days, then grinding and sifting the powder through an 80-mesh sieve. In the second group of experiments described, green tissue was ground in a mortar

FIG. 7.—Fasciated sunflowers produced by irradiating 5-day old seedlings with 5 E. (left) and 10 E. (right); strap-shaped condition of stem apparent in both specimens.

with an excess of calcium carbonate, and then washed quantitatively into the shaking bottle with 15 cc. of distilled water. Care was taken to grind the tissues each time to a comparable state of fineness. When the bottle with its contents had reached the temperature of the bath, 5 cc. of dioxygen was added and the whole shaken for ten minutes. The majority of determinations were run in triplicate, the numbers reported being the average of these determinations. Whenever possible a small enough amount of plant material was used, so that 18–20 cc. of oxygen was liberated in ten minutes, since recent

experiments (32) have shown that this adjustment gives the most satisfactory results.

1. CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS EXPOSED TO 4, 10, AND 15 E.—There is a depression of catalase activity in seedlings whose seeds have been exposed to the action of X-rays. This is less marked in older seedlings and is accompanied by greater growth. In cases cited earlier in this paper, doses not causing death of a plant usually showed only transitory effects upon growth. Table V gives the results of catalase determinations made with dried powder of seedlings grown for the stated number of days, and then

TABLE V

CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS IRRADIATED WITH 4, 10, AND 15 E.; DETERMINATIONS MADE ON SMALL QUANTITIES OF AIR-DRY POWDER

DOSE	CONDITION OF SEED	AGE OF PLANT (DAYS)	WEIGHT OF POWDER IN (GM.)	CC. OF O_2 LIBERATED IN MINUTES					O_2 PER GM. AIR-DRIED WEIGHT (CALCULATED)
				1	2	3	5	10	
4 E.....	59% water	5	0.015	8.6	13.0	16.9	20.2	25.01	1840
Control..	59% water	5	0.015	14.2	17.7	22.3	28.2	35.06	2337.33
15 E.....	Air-dry	5	0.015	9.7	14.6	17.7	21.4	25.55	1704
Control..	Air-dry	5	0.015	11.8	18.7	22.32	27.0	31.89	2126
10 E.....	56.5% water	13	0.01	8.27	13.51	16.09	19.96	21.55	2155
Control..	56.5% water	13	0.01	10.89	16.66	20.00	24.27	29.28	2928

prepared as already stated. The 5-day old seedlings were grown in sphagnum, while those given a dose of 10 E. and examined at the end of thirteen days were grown in soil. The latter seem to have recovered from the effects of the dose, since the catalase activity became almost equal to that of the control.

2. CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS EXPOSED TO KILLING DOSE.—Soaked seeds with a water content of 58.7 per cent were exposed to a dosage of 40 E. Seedlings from these developed to a total height of 1–3 cm. They remained green for some time without increasing in size, and then died. Seedlings for this experiment were grown in sphagnum, and at intervals, as stated in the experiments, were used in determining catalase activity. The 53-hour seedling was found to contain too much catalase for the entire seedling to be used in the determination, hence one-half or one-

fourth of the seedling, containing all representative parts, was used in all determinations except the first one. Oxygen liberated per gram absolute dry weight was used as a basis for comparison of the catalase activity of seedlings selected at successive intervals of 48 hours, and controls of the same age. Table VI gives results from this series of experiments, and fig. 8 gives diagrams representing determinations made. The activity of the control is represented by clear rectangles, while the black rectangles show determinations made on irradiated material.

TABLE VI

CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS RECEIVING A VERY HEAVY DOSE (40 E.); DETERMINATIONS MADE AT 48-HOUR INTERVALS

AGE AND LENGTH OF SEEDLING	WEIGHT OF GREEN MATERIAL (GM.)	CC. OF O_2 LIBERATED IN MINUTES					O_2 PER GM. GREEN WEIGHT (CALCULATED)	O_2 PER GM. ABSOLUTE DRY WEIGHT (CALCULATED)
		1	2	3	5	10		
5-hour seedling....	0.0688	2.6	5.1	7.1	10.0	14.2	206.35	324.7
Control.....	0.0606	1.8	3.4	4.8	6.7	8.2	135.3	210.7
53-hour seedling... (1 cm.).....	0.019	5.5	10.0	13.5	17.8	23.5	1236.84	3283.0
Control (2.5 cm.)..	0.027	11.4	13.7	21.9	26.2	29.8	1103.7	4405
101-hour seedling (2 cm.).....	0.0325	7.8	13.0	16.7	22.1	27.9	858.5	3430
Control (12 cm.)...	0.1051	17.0	29.1	36.5	49.5	51.8	494.76	3320
149-hour seedling (2.4 cm.).....	0.0541	3.74	7.2	9.6	13.3	18.7	534[illegible].6	1540
Control (18 cm.)...	0.0966	7.5	12.7	17.5	25.9	29.5	8220.1	2106
177-hour seedling.. (2.6 cm.).....	0.0866	5.0	8.4	10.8	14.73	21.15	244.2	1572
Control (21 cm.)...	0.1921	5.6	9.4	10.8	16.8	23.3	121.3	2369

The preceding experiments showed consistently lower catalase activity in irradiated material than in the control seedlings. It may be concluded, therefore, that irradiation causes a depression in catalase activity.

IV. Effect on respiration

After finding decreased catalase activity in seedlings whose seeds had received a heavy dose of X-rays, one would naturally inquire as to the effect of irradiation of seeds upon respiration of their seedlings. No previous experimentation seems to have been done with the respiration of irradiated plants. One investigator (7) found that canaries used an increased amount of oxygen immediately after ex-

posure to X-rays, but after one hour metabolism was much lower than it had been before.

In the two following experiments, comparisons were made between respiration of normal seedlings and seedlings of the same age whose seeds had been exposed to heavy doses. The amount of CO_2 liberated by the seedlings was used as an index to respiratory activity.

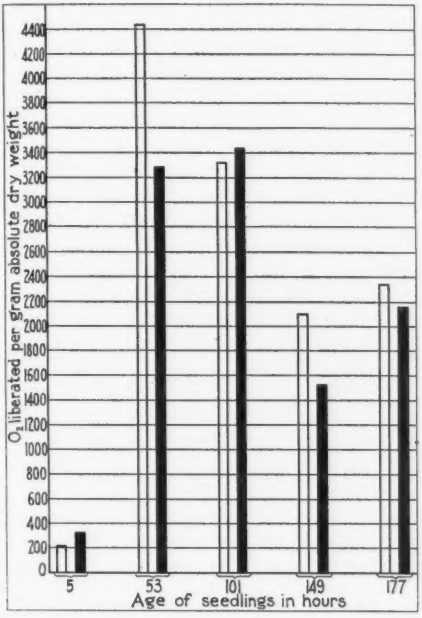

FIG. 8.—Diagrams representing catalase activity of seedlings from heavily irradiated seeds (black rectangles) as compared with that of controls (clear rectangles); diagrams based on data in table VI.

Helianthus seeds having a water content of 58.7 per cent were exposed to a dose of 40 E. A few hours after irradiation, they were placed on moist cotton in a type of respirometer devised and described by OTA (**30**). By means of this apparatus only CO_2-free air is admitted to the respiratory chamber. The atmosphere of the chamber is exposed to normal NaOH, which occupies a tube placed just below the respiratory chamber. The respirometers were placed in a Freas water bath, the temperature of which was kept at 20° C. After each 24-hour period, the amount of CO_2 given off by the seedlings was determined by the double titration method of BROWN and ESCOMBE (**8**). Table VII shows the average results, from two series of determinations, of the amount of CO_2 expired from twenty seedlings.

Seedlings from heavily irradiated seeds apparently lack the ability which an ordinary seedling has to take up water. It seems possible

that the irradiation affects the permeability of the membranes in such a way that absorption is retarded. Absolute dry weight determinations showed that seedlings from irradiated seeds had a much higher percentage of dry weight than the controls. For this reason a

TABLE VII

AMOUNT OF CO_2 LIBERATED DAILY PER 20 SEEDLINGS FROM SEEDS DOSED WITH 40 E.; CO_2 FROM CONTROLS ALSO GIVEN

AGE OF SEEDLINGS (DAYS)	MILLIGRAMS OF CO_2 PER 20 SEEDLINGS PER DAY	
	Irradiated	Controls
1	14.3	18.1
2	23.2	26.5
3	24.2	28.32
4	23.1	27.5
5	22.2	37.0
6	22.2	35.2
7	22.0	30.0
8	22.0	.....
9	17.6	25.3

determination was made of the amount of CO_2 expired per gram of absolute dry weight. Seed treatment was as described, except that the dose given was 24 E., an amount sufficient to stop growth after a length of 1–3 cm. had been attained. Average results from two series of experiments are recorded in table VIII.

TABLE VIII

AMOUNT OF CO_2 EXPIRED BY SEEDLINGS FROM IRRADIATED SEEDS

AGE OF SEEDLING (HOURS)	MILLIGRAMS CO_2 PER GM. ABSOLUTE DRY WEIGHT PER HOUR	
	Irradiated	Control
78	1.742	3.043
102	3.369	4.317

These results indicate that depressed respiration accompanies inhibition of growth caused by a dosage of X-rays varying from 24 to 40 E. Normally seedlings which are deprived of a proper food supply lose considerable dry weight through the oxidation processes. That

seedlings from irradiated seeds showed a higher percentage of dry weight present would indicate that oxidation was being retarded and the CO_2 output decreased. Others (**6, 12, 35**) have found parallel behavior between catalase activity and respiration in normal seedlings. Results here offered give added proof of correlation between the two processes, for both are depressed by X-rays.

V. Effect on oxidase activity

Oxidase activity was neither depressed nor increased in seedlings from irradiated seeds. These results may be contrasted with those of BUNZELL (**10, 11**), who found that seedlings dwarfed by under or over supply of water had oxidase activity increased.

A study was made of leaves which showed anomalies of form resulting from irradiation. These did not exhibit increased oxidase activity as do leaves showing physiological disturbances due to diseases. Abundant evidence (**10, 11**) has been found of increased oxidase action in leaf tissue of plants in cases of mosaic disease of tobacco, curly top of sugar beet, and diseases of spinach. WELS (**38**) believes that the growth-checking effects of X-rays are not due to an influencing of oxidation processes in cells of yeast, bacteria, and some animal cells. Others (**27**) have found that the oxidase system of leucocytes was not influenced by the rays.

1. OXIDASE ACTIVITY OF ENTIRE SEEDLINGS FROM HEAVILY DOSED SEEDS.—Oxidase activity was found to be unchanged in seedlings from seeds which had been irradiated with a dose of 24 E. Seeds with a water content of 58 per cent were exposed to that dose. After growth for five days in sphagnum, the young plants were dried before an electric fan, powdered to pass through a sieve of 80 meshes to the inch, and the powder then thoroughly dried in a vacuum desiccator over calcium chloride.

Determinations were made in the simplified apparatus described by BUNZELL (**9**), the principle of which involves the measurement of the rate of oxygen absorption as determined by measuring changes of pressure within the reaction flask. The oxidizing agent employed was 1 per cent pyrocatechol. The rate of shaking was five complete revolutions in approximately three seconds. A definite amount of dried powder was placed in the long arm of the apparatus by means of a funnel and a camel's hair brush. To this 5 cc. of distilled water

was added; 1 cc. of 1 per cent pyrocatechol was added to the short arm. The apparatus and its contents were allowed to stay at the temperature of the experiment for thirty minutes, before the apparatus was closed and shaking begun. Table IX shows the results obtained with use of absolutely dry powder from plants whose seeds had received a dose sufficient to cause death of seedlings within a few days after germination. The table shows that there is no significant difference between the activity of the irradiated material and that of the control.

2. OXIDASE ACTIVITY OF ABNORMAL LEAVES FROM IRRADIATED SEEDS.—The material for use in this experiment was collected from

TABLE IX

MANOMETER READINGS OBTAINED BY USE OF 0.02 GM. POWDER FROM 5-DAY SEEDLINGS WHOSE SEEDS HAD RECEIVED DOSE OF 24 E.; DURATION OF EXPERIMENT 1.5 HOURS

No. of determination	Temperature (°C) at time of measurement	Manometer readings expressed in cm. of mercury	
		Plants from irradiated seeds	Normal plants
I.....	28.0	2.7	2.6
II.....	28.0	2.4	2.4
III.....	28.5	2.55	2.5
IV.....	28	2.3	2.3
V.....	28	2.3	2.3

plants whose seeds had been irradiated with doses light enough to avoid death. Leaves showing incipient fasciation, or which were abnormal in other ways, were collected, dried, powdered, and stored in a desiccator. Control leaves were treated in the same manner. Table X shows results of these determinations, and again only a slight difference is apparent in oxidase activity of abnormal leaves and that of control leaves.

From the data presented in tables IX and X, it is evident that inhibition of growth is not always, as has been maintained, accompanied by increased oxidase activity. There is approximately no difference in the oxidase activity of equal quantities of dry powder derived from retarded seedlings and that from control seedlings; neither does the oxidase activity of leaves deformed by the action of X-rays differ materially from that of normal leaves.

Oxidase appears to be an exceedingly stable system, and an amount of irradiation which inhibits growth after the seedling has attained a height of 1–2 cm. does not affect oxidase activity.

VI. Histological investigation

1. STEMS OF MATURE PLANTS AND OF 9-DAY OLD SEEDLINGS.—One character which was most noticeable in mature plants from irradiated seeds, and never apparent in the controls, was the roughened, dark brown appearance of the stem in the hypocotyl region. When examined microchemically, it was found that the epidermis of the irradiated material, which was broken and irregular in outline, contained considerable suberin, while little or none was present in the control.

TABLE X

MANOMETER READINGS OBTAINED BY USE OF 0.02 GM. OF POWDER FROM ABNORMAL LEAVES OF IRRADIATED SEEDS; DURATION OF EXPERIMENT 1.5 HOURS

No. of determination	Temperature (°C) at time of measurement	Manometer readings expressed in cm. of mercury	
		Leaves showing anomalies	Normal leaves
I.....	27.50	0.50	0.45
II.....	28.70	0.45	0.60
III.....	28.75	0.40	0.50

Cross-sections were cut through the hypocotyl region of mature plants whose seeds had received doses of 8, 9, and 10 E. respectively. These were made midway between the emergence of the first lateral root and the point of attachment of the cotyledons. When compared with cross-sections of the control in the same region, there was noted a great increase in the amount of xylem in the irradiated specimens, and a corresponding decrease of pith cells. Table XI shows measurements of cross-section of stem taken midway between the emergence of the first lateral root and the point of attachment of the cotyledons. Each measurement given is the average of three taken on the same section. Measurements are recorded from sections of two control plants, and from two plants whose seeds were subjected to a dose of 8 E. Of those given 9 and 10 E., only one plant each was used for measurement of sections.

From results given, it is seen that while there is no appreciable increase in the amount of cork in the control stem, the corky layer of the irradiated material occupies 8–10 per cent of the whole width. The cortex, which in the control stems makes up approximately 22 per cent of the diameter of the section, occupies only 9.5–18.7 per cent of the whole in stems whose seeds were X-rayed. There is a marked difference in the amount of xylem present in the irradiated material and in the control stems. In the former the xylem constitutes 57.7–69.8 per cent of the total diameter, but only about 38

TABLE XI

MEASUREMENTS OF CROSS-SECTION OF STEM TAKEN MIDWAY BETWEEN EMERGENCE OF FIRST LATERAL ROOT AND POINT OF ATTACHMENT OF COTYLEDONS; EACH MEASUREMENT IS AVERAGE OF THREE TAKEN ON SAME SECTION

DOSE	WIDTH OF PITH IN μ	PERCENTAGE OF WHOLE	WIDTH OF XYLEM IN μ	PERCENTAGE OF WHOLE	CORTEX IN μ	PERCENTAGE OF WHOLE	CORK LAYER IN μ	PERCENTAGE OF WHOLE
Control (a)	3066	37.2	1566	38.0	1017	24.8	.....	.....
Control (b)	3417	41.4	1600	38.7	817	19.8	.....	.....
Average	3241	39.3	1583	38.3	917	22.3	.....	.....
8 E. (a)	1066	11.3	3217	68.6	567	12.1	367	8.0
8 E. (b)	907	8.4	3833	71.1	650	12.0	450	8.5
Average	986	9.8	3525	69.8	608	12.0	408	8.2
9 E	1133	16.6	2217	65.0	325	9.5	300	8.9
10 E	10000	13.7	2100	57.7	683	18.7	354	9.9

per cent in the control. In control stems, the diameter of the pith is at least three times that of stems whose seeds received a moderate amount of irradiation. Others (**1, 29**) also have noted the increase of xylem and cork and the decrease of pith which accompanied irradiation. Fig. 9 shows diagrammatically the relative proportions of the various regions of the stem in half sections taken from a control hypocotyl, and from a seedling whose seed had received a dosage of 10 E.

The character of the elements present in the cross-sections of the stem differs in irradiated material from that of the control. In the former the pith cells from the same regions are much smaller in diameter, and are slightly thicker walled. The irradiated material has smaller and more compactly arranged xylem cells. Fig. 10 shows

drawings of a few cells from the center of the pith regions of stems which are being compared. Fig. 11 represents the protoxylem and a portion of primary xylem in the hypocotyl region of a mature control plant, and of one whose soaked seeds received a dose of 10 E. The wood elements in the irradiated specimens are seen to be of smaller dimensions than are those of the control.

Paraffin sections of hypocotyl of seedlings nine days old were examined to find whether plants derived from seeds which had been exposed to a dose of 10 E. showed any differences in their anatomical structures from controls of the same age. The most striking differences seemed to be in the amount and character of the xylem. The irradiated material had a very marked increase in the number of xylem cells. Instead of showing fibrovascular bundles somewhat oval in shape, as is characteristic of bundles in young control stems, the xylem region of each bundle was much extended in width (tangentially). The number of distinct bundles in the control stems examined was ten. The xylem varied in width from 80 to 130 μ. The stem of the irradiated material, however, showed but seven distinct fibrovascular regions. In one portion the xylem tissue appeared as a continuous mass, and looked as if three or more vascular bundles had fused. The individual areas of xylem varied in width from 80 to 560 μ. The total circumferential width of xylem of the

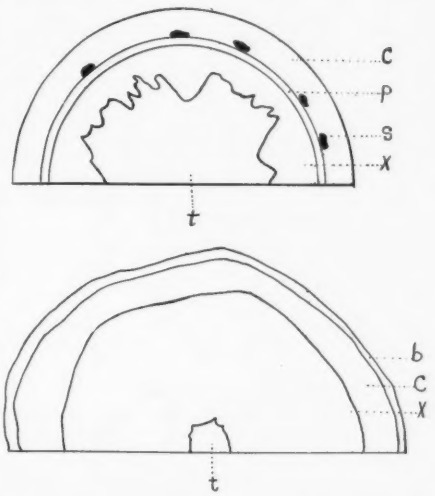

Fig. 9.—Diagram of transverse section of stem through hypocotyl region of mature plant: above, control; below, from plant whose seeds had received dose of 10 E. (*b*, cork; *c*, cortex; *s*, hard bast; *p*, phloem; *x*, xylem; *t*, pith); phloem of irradiated material not distinguishable, as it was injured by pressure of xylem; no cork formed in control stem.

hypocotyl from irradiated material amounted to 1530 μ, a width approximately 45 per cent greater than that of control tissue.

The individual xylem cells in the hypocotyl of irradiated specimens showed a great similarity of size. In the control small cells measured approximately 5×7 μ, the largest cells 40×35 μ. In the irradiated material the cells were of more uniform size. The average of many cells was approximately 13.5×11.5 μ.

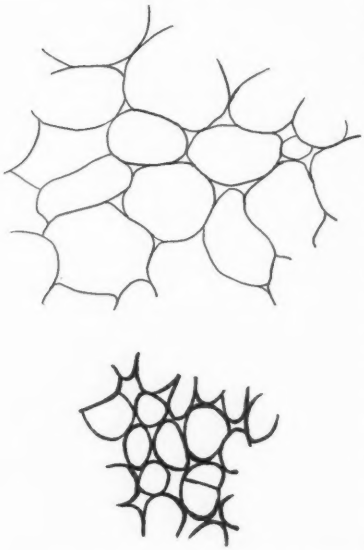

FIG. 10.—Cells from center of pith region of mature stem: above, control; below, from plants whose seeds had been irradiated with dose of 10 E.; ×150.

2. RADICLES OF 6-DAYS OLD SEEDLINGS.—The diameter of the control radicle at a distance of 200 μ from the base of the root cap was 400 μ in diameter. The appearance of the cells was similar to that of the usual young root cells. The shape was rectangular; the cells, practically all of the same size, were thin walled and well filled with protoplasmic contents. The nucleus was large in proportion to the size of the cell. Cells in this meristematic region were measured, and the average size of ten cells found to be approximately 13.5×15 μ.

The radicle from irradiated material was only about one-half as thick as the control root. The cells in a region comparable with that studied in the control showed an entirely different appearance. They indicated that all growth had ceased, and the cells seemed to have reached the stage of cell enlargement. The cells were elongated; many of them had lost their protoplasmic contents entirely, others showed great vacuolation. KOMURO (**26**) records similar results from his study of the root tip of *Vicia Faba*. In marked contrast to the meristem of the control, where in these preparations a nucleus was evident

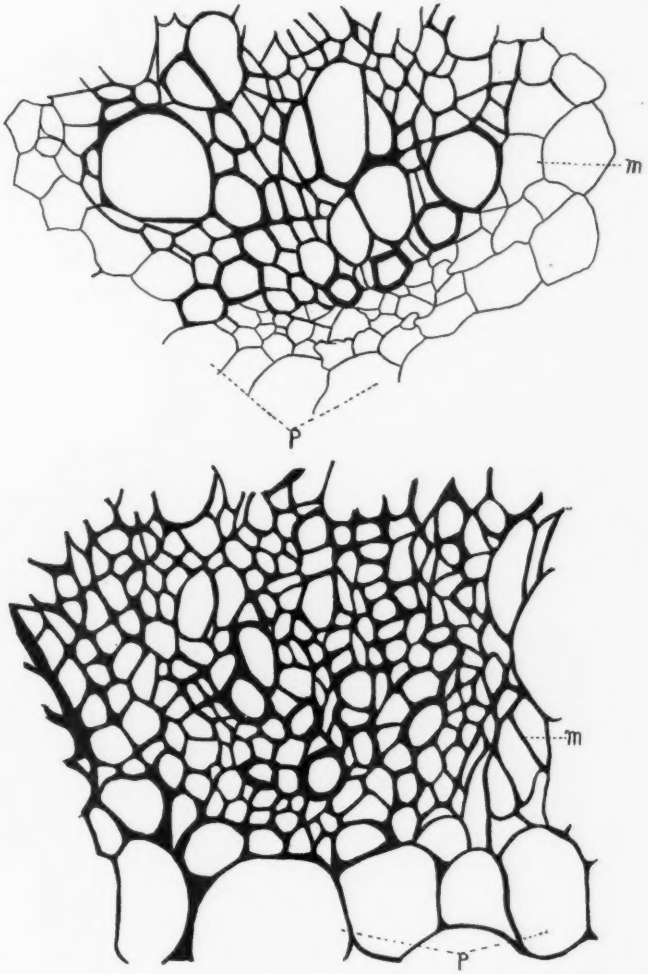

FIG. 11.—Above, primary xylem from mature stem of control (*p*, pith; *m*, medullary ray cells); below, primary xylem from mature stem of plant whose seed had received dose of 10 E. (note smaller dimensions of wood elements, thickened walls of pith cells (*p*), and of medullary ray cells (*m*), as compared with those of control stem); ×600.

in every cell, the meristem of the radicle of the irradiated material showed but an occasional nucleus. In the same region of the root as that examined in the control, the average size of ten cells measured was 26×46 μ, or twice the cross-section area of the control.

Discussion

In this investigation the plants were allowed to grow to maturity, so that not only the immediate influence of irradiation was noted, but any later after-effects as well. In summarizing the results of investigators who have studied the influence of X-rays on seeds and seedlings of various kinds, GELLER (**15**) made the criticism that most of the observations were not carried on long enough to determine whether the effect on plants is merely a transitory one, or whether it also exerts an influence on the total amount of growth.

Preliminary experiments showed that no immediate visible effects resulted when plants were given a light dose (4 E. and below). These plants at maturity, nevertheless, were shorter and weighed less than the controls. The period required for blossoming was also shortened. When a heavy dose (20–30 E.) was given, growth stopped after the hypocotyl had developed to an average length of 1.5 cm. The tip of the root showed a brown coloring, and, in the great majority of cases, no sign of a growing stem tip was visible.

Germination of seeds is not hastened by irradiation, but the percentage of seeds which germinate is slightly reduced. Irradiated seeds showed a decrease of 6.5 in percentage of germination. Of 520 seeds irradiated 88.2 per cent germinated, while of 362 seeds used as controls 94.7 per cent germinated.

Soaked seeds exposed to medium doses (5–10 E.) showed, during the first three weeks, a checking of growth which was followed by a transitory acceleration of development. This temporarily increased growth was never sufficient to cause an increase over that of the controls in the total dry weight of mature plants from irradiated seeds or seedlings.

Different seeds of the same species may react variously to the same dosage of X-rays, as shown in one experiment where *Helianthus* plants grew eight times as tall as others from seeds irradiated similarly and exposed to identical environmental conditions. Experi-

ments with animals have shown that different individuals of the same species often exhibit considerable variation in their susceptibility to X-rays.

Fasciation is a fairly constant character produced by X-rays, as this occurs not only in irradiated sunflowers but in irradiated tomato and cosmos plants as well. By irradiating seeds and seedlings, fasciations of stems, leaves, and flowers were induced. In some cases these appeared as early as the fourth week of growth. The stems became flattened, showing bifurcations or splittings. These branches usually divided again. This branching of stems was very noticeable because the sunflowers used were of a non-branching type. Of the eighteen groups (consisting of eighty-one controls) which were grown to maturity, all were strictly one-stemmed individuals. Fasciations in leaves were shown by changes in number, position, size, and shape. Phyllotaxy was distorted and the number of leaves was commonly increased. Fasciation of the flower head was present in many forms: fusion in the involucral region, forking of the stalk below the involucre with two distinct heads resulting, and the appearance of triradiate heads.

A frequent explanation for fasciations is that they are induced by changed internal conditions. Care was taken to subject controls, irradiated seeds, and irradiated seedlings to the same environmental conditions in the greenhouse. This could not insure absolutely equal internal conditions of nutrition, however, and it is possible that the plane of nutrition is much lower in the experimental material than in the controls.

It has been maintained (**40, 41**) that irradiations from either X-rays or radium cause irreversible changes in the protoplasm of the cell. This could hardly have occurred in the protoplasm of the sunflower cells, for, with light or medium doses, apparently normal growth takes place after recovery from the period of growth-checking.

Seeds in air-dried condition were found to be less easily influenced by the action of X-rays than were those with a water content above 50 per cent. Seedlings beginning active growth were more sensitive than resting or soaked seeds. These results are in accord with those of practically all other investigators in this field. Animal

cells also have been found by others (**2, 3**) to be more susceptible to X-rays during mitosis than during rest.

Catalase activity is lessened in young seedlings whose seeds have been exposed to medium doses of irradiation. With older seedlings which are beginning to show recovery from the checking effects of irradiation, the activity is almost equal to that of the control. With seedlings whose seeds were exposed to killing doses of X-rays, a greater depression of catalase activity was noted.

Depressed respiration accompanies the marked inhibition of growth caused by heavy irradiation. Soaked seeds given heavy dosage were placed on moist cotton in a respirometer which was supplied with CO_2-free air. The amount of CO_2 liberated per unit of absolute dry weight was used as an index to respiratory activity. When compared with the CO_2 output of the control, that of seedlings from irradiated seeds showed a decided decrease.

Oxidase activity remains unchanged in seedlings grown from irradiated seeds. Checking of growth accompanying heavy irradiation must differ from that associated with dwarfing due to excessive watering, drought, disease, or some unknown agencies.

Histological study of the meristematic region of the tip of radicles from irradiated seeds showed marked differences in appearance from that of the controls. Cells from irradiated material showed elongation, great vacuolation or entire absence of protoplasm, and the absence of nuclei from many cells. The average size of the cells in the meristematic region of the radicle from irradiated material is twice the cross-section area of the control.

Increase of xylem with a corresponding decrease of pith cells is apparent in the hypocotyl region of mature plants whose seeds have been irradiated. The character of the elements differs in the two stems. In cross-sections from the hypocotyl regions of irradiated material, the pith cells are much smaller in diameter and are thicker walled than in control stems. The elements of the xylem in the former are much smaller in diameter, and arranged more compactly. Even in seedlings nine days old, a striking difference is shown in the amount and character of xylem. There is here also a much greater percentage of xylem in the irradiated specimens, with individual cells showing a similarity in size, rather than the wide diversity in

size of cells which is typical for xylem of young *Helianthus* stems. Epidermis on the lowest internodal regions of mature plants from seeds which had received medium doses of irradiation was noticeably dark brown in color, the cells irregular in outline, and the material when tested microchemically was found to be impregnated with considerable suberin. Tests on the epidermis of the control showed no suberin present.

Summary

1. Irradiation of soaked seeds of *Helianthus annuus* causes a slight decrease in percentage of germination. The average total growth of the plants is less, and the average time of blossoming is shortened, even with doses so light that there are no immediate visible effects. A checking effect, proportional to the doses, is evident during the first three weeks of growth after seeds have been irradiated with medium doses. On unfolding, the leaves present a peculiar pitted or mosaic appearance, which seems to be due to an unequal distribution of chlorophyll, when medium or heavy doses are given the seeds. Heavy dosage does not stop development of soaked seeds until the cotyledons have been partially or wholly pushed through the soil. The root tip becomes brown in color and no growing stem tip appears.

2. Fasciations of stems, leaves, and flowers are induced by irradiation of seeds and seedlings with medium doses. Stem fasciations commonly took the form of flattening and dichotomous splitting of the main stem, which showed the presence of many blossoming branches instead of a single flower head. Fasciation of the flower head was represented by fusion in the involucral region and by forking of the stalk below the involucre, with two or three distinct heads resulting. Many leaf abnormalities,—ligulate leaves, altered phyllotaxy, and incipient fasciation, which marked earlier periods of growth, often disappeared before maturity.

3. Catalase activity is depressed in young seedlings whose seeds have been exposed to medium doses of X-rays. With older seedlings which are beginning to show recovery, however, there is but slight reduction in activity noticed.

4. Depressed respiration is associated with stunted growth when this accompanies heavy irradiation. The amount of carbon

dioxide liberated from irradiated seedlings is less than that from controls similarly treated.

5. Normal oxidase activity is found in seedlings whose growth has been inhibited by heavy doses of X-rays.

6. Cells of the radicle tip from seedlings whose growth has been inhibited by heavy irradiation show elongation, great vacuolation or entire absence of protoplasm, and the absence of nuclei from many cells. The average size of the cells in the meristematic region of the radicle tip of irradiated material is greatly increased over that of the control. Xylem is increased at expense of the pith, and there is a greater suberin development in the hypocotyl regions of mature plants from irradiated seeds than in those of the control. The increase in vascular tissue in irradiated material is apparent even in seedlings but nine days old.

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POSSIBLE ETIOLOGICAL RÔLE OF PLASMODIOPHORA TABACI IN TOBACCO MOSAIC

G. K. K. LINK, P. M. JONES, AND W. H. TALIAFERRO[1]

Introduction

The recent papers of ECKERSON[2] and JONES[3] have again compelled consideration of the possible association of protozoa and mosaic of potato, tobacco, and tomato plants. Miss ECKERSON reports the discovery of actively moving flagellated and amoeboid bodies, as well as "spores" in the mesophyll cells of tomato plants affected with mosaic. JONES reports success in culturing a mycetozoan (named by him *Plasmodiophora tabaci*) from tobacco tissues affected with mosaic-like symptoms, and the development of identical symptoms following the inoculation of healthy plants with such cultures. He also reports that he was able to find the plasmodial stage of the protozoan in tissues of tobacco plants with similar symptoms, and that healthy plants were entirely free from it.

The present investigation was undertaken to ascertain whether there is a constant association of JONES' organism with tobacco mosaic, and whether it plays any rôle in the etiology of the disease.[4] The following lines of experiments have been carried out: (1) Tissues from healthy and diseased tobacco plants were cultured, to determine whether a correlation exists between the mycetozoan and mosaic. (2) Healthy tobacco plants were inoculated with cultures of *P. tabaci* obtained originally from healthy and diseased tissues, to ascertain whether such cultures carried the virus of tobacco mosaic.

[1] Joint contribution from the Department of Botany and the Department of Hygiene and Bacteriology of the University of Chicago.

[2] ECKERSON, SOPHIA H., An organism of tomato mosaic. BOT. GAZ. **81**: 204–209. 1926.

[3] JONES, P. M., Structure and cultural history of a mycetozoan found in tobacco plants with mosaic-like symptoms. BOT. GAZ. **81**: 446–459. 1926.

[4] As work of this character involves different techniques, it was thought best to carry out the experiments with the cooperation of several men of different training. Of the three authors, P. M. JONES is the discoverer of the mycetozoan, G. K. K. LINK is a plant pathologist, and W. H. TALIAFERRO is a protozoologist.

(3) Juice of diseased plants was filtered through diatomaceous and porcelain filters, the filtrate cultured, and also inoculated into young tobacco plants to ascertain whether *P. tabaci* can be cultured from known infective filtrates. (4) Cultures of *P. tabaci* were similarly filtered and the filtrates cultured to test the filterability of the mycetozoan. The infectivity of such filtrates was also tested by suitable inoculations.

The virus used in these experiments was obtained from tobacco plants kindly furnished us in November, 1925, by Dr. J. C. JOHNSON of the University of Wisconsin. In these experiments, two varieties of tobacco were used, Connecticut Broad Leaf and Kentucky Mammoth.

I. Culture of tissues

In carrying on the culture work the methods used by JONES (*loc. cit.*) were followed, with modifications as the work progressed. The cultures were made in Erlenmeyer flasks with necks large enough to permit passage of cover glasses, which were used in examinations for presence of the organism. At first Knop's solution was used, but later it was found that distilled water was equally efficacious. The flasks containing the medium and cover glasses were sterilized in an autoclave at 15 pounds pressure for 45 minutes. Young leaves were used in most of the cultures, but stems and roots were also employed. The tissues were so manipulated that there was no possible contamination from one lot to another during handling. Immediately after collection each leaf or part of leaf, stem, or root was rinsed in water, washed in a solution of mercuric chloride (1:1000) for 1–3 minutes, and then rinsed in sterile water. Lots DA, DB, and DC of diseased tissues, and lots HB, HC, and HF of healthy tissues were prepared in this manner. Later tissues were collected similarly but were not washed in mercuric chloride. These were lots DD, DE, and DF of diseased tissues, and HE and HF of healthy tissues.

The cultures were incubated at room temperatures (the range being 15°–25° C.); some in diffuse light and some in darkness. They were examined the first time after 15–20 days, and then at intervals of a week. For examination, a cover glass was removed with a flamed inoculation needle and then mounted face up on a slide. The results of these experiments are assembled in table I. No healthy plants

were cultured in the first lot. In samples DA 1–5 of January 30, the leaves were cut so that each culture contained only a portion of a leaf. Lots DA and DB of March 20, DC of May 15, DE of May 26, and DF of August 3 were run against the controls HB, HC, HE, and HF respectively. In these experiments only entire leaves were used, while the cultures of roots and stems consisted of tissue fragments.

TABLE I

CULTURES OF DISEASED AND HEALTHY TISSUES FOR PROTOZOA

CONDITIONS OF EXPERIMENT	PLANT	ORGAN	DATE OF MAKING CULTURE	DATE OF CLOSING CULTURE	NO. OF CULTURES	NO. OF CULTURES WITH ORGANISMS
Diseased tissues, washed in water, placed in mercuric chloride, and rinsed in sterile water	DA 1–5	Portion of leaf	January 30	February 19	16	9
	DA 5	Root	January 30	February 19	2	1
	DA 6–7	Stem	January 30	February 19	2	2
	DA 7	Petiole	January 30	February 19	1	1
	DA 1–5	Leaf	March 20	May 4	10	10
	DB 2,6,7	Leaf	March 20	May 24	9	9
	DB 1,6,6,9	Root	March 20	May 24	6	6
	DB 2,4,6,8	Stem	March 20	May 24	7	7
	DC 1–5	Leaf	May 15	June 17	25	13
					Total..78	58
Diseased tissues, washed in sterile water only	DD 1–3	Entire top of plant	May 17	May 27	3	3
	DE 1–10	Leaf	May 26	June 17	10	8
	DF 1–26	Leaf	August 3	August 26	26	21
					Total..39	32
Healthy tissues, washed in water, placed in mercuric chloride, rinsed in sterile water	HB 1–10	Leaf	March 20	May 4	10	1
	HC 1–25	Leaf	May 15	June 17	25	0
	HF 1–5	Leaf	August 3	August 26	5	3
					Total..40	4
Healthy tissues washed in sterile water only	HE 1–11	Leaf	May 26	June 17	11	10
	HF 1–5	Leaf	August 3	August 26	5	2
					Total..16	12

A consideration of the data in table I indicates that there is no significant difference between the results obtained when healthy and diseased tissues were cultured, provided the tissues were not washed (sterilized) in mercuric chloride. On the contrary, there was a marked difference between healthy and diseased lots provided the tissues were first washed (sterilized) in mercuric chloride. Thus, out of a total of 39 cultures from diseased plants not washed in mercuric chloride, 32 showed JONES' organism. Similarly, from 16 cultures

of healthy plants not washed in mercuric chloride 12 showed it. On the other hand, from a total of 78 individual cultures from diseased tissues which were washed (sterilized) in mercuric chloride 58 showed

TABLE II

INOCULATIONS WITH CULTURES FROM DISEASED PLANTS FOLLOWED BY DEVELOPMENT OF MOSAIC

DATE OF INOCULATION	SOURCE OF CULTURE	PROTOZOAN IN CULTURE	INOCULATED PLANTS			CONTROLS		DATE OF CONCLUSION OF EXPERIMENT
			Total	Diseased	Healthy	Diseased	Healthy	
February 13	DA 4 Leaf....	None	3	2	1	0	3	March 1
	DA 4 Leaf....	Flagellates	3	3	0	0	3	
	DA 4 Leaf....	Cysts	3	3	0	0	3	
	DA 5 Root...	Flagellates	3	3	0	0	3	
	DA 5 Tip of plant.	Amoebae	3	3	0	0	3	
March 16	DA 1 Leaf....	Cysts, "parasites"	14	9	5	1	6	April 7
March 19	DA 1 Leaf....	Cysts, "parasites"	20	20	0	0	15	May 5
	DA 3 Leaf....	Flagellates	15	15	0	0	15	
April 7	DA 2 Leaf....	Cysts, "parasites"	6	6	0	0	4	May 5
	DB 1 Leaf....	Amoebae, flagellates	6	2	4	0	4	
	DB 6 Root...	No organisms	6	1	5	0	4	
	DB 6 Root...	Flagellates and ciliates	6	2	4	0	4	
May 5	DA 4 Leaf....	Cysts	5	1	4	0	4	June 2
	DB 7 Leaf....	No organisms	6	2	4	0	8	
	DB 7 Leaf....	Cysts, "parasites"	5	4	1	0	4	
	DB 7 Leaf....	Cysts	5	3	2	0	4	
	DB 7 Leaf....	Cysts, "parasites"	5	1	4	0	4	
	DB 8 Leaf....	Flagellates and ciliates	6	2	4	0	4	
June 6	DD 1 Top of plant.	Amoebae, flagellates, cysts	3	1	2	0	8	June 24
	DC 1 Leaf....	No organisms	3	1	2	0	8	
	DE 2 Leaf....	Flagellates	2	2	0	0	8	
	DE 3 Leaf....	Flagellates	2	1	1	0	8	
June 19	DE 5 Leaf....	None	6	6	0	0	2	July 17
	DE 6 Leaf....	None	6	6	0	0	2	
June 20	DE 4 Leaf....	None	5	1	4	0	4	July 17
	DE 5 Leaf....	None	5	4	1	0	4	
	DE 6 Leaf....	None	7	5	2	0	4	
		Total.....	159	109	50	1	143	

organisms; but in 40 cultures from similarly treated healthy plants only 4 showed organisms.

II. Inoculation experiments

The inoculation experiments were made mainly with cultures reported in table I, and were designed primarily to ascertain whether the power to produce mosaic could be separated from the presence of *P. tabaci*, or whether the two were always associated. We were

greatly aided by the discovery, as already noted in the previous section, that typical cultures of *P. tabaci* could be obtained from healthy plants which had never shown evidences of the disease. As inoculations were made with cultures containing the organisms, both from diseased and healthy tissues, and with cultures from diseased tissues which did not contain the organisms at the time of inocula-

TABLE III

INOCULATIONS WITH CULTURES FROM DISEASED PLANTS NOT FOLLOWED BY DEVELOPMENT OF MOSAIC

Date of inoculation	Source of culture	Protozoan in culture	Inoculated plants			Controls		Date of conclusion of experiment
			Total	Diseased	Healthy	Diseased	Healthy	
February 12	DA 5 Root...	None	3	0	3	0	3	March 1
May 5	DA 4 Leaf....	Amoebae, flagellates	4	0	4	0	4	June 2
	DA 4 Leaf....	Cysts (cultures 10 days old)	5	0	5	0	4	
	DB 9 Stem (1 year old)..	Amoebae	3	0	3	0	4	
May 26	DD 1 Entire top...	Amoebae, cysts, ciliates, and ciliate cysts	4	0	4	0	10	June 24
	DD 2 Entire top...	Amoebae, flagellates	4	0	4	0	10	
	DD 3 Entire top...	Flagellates, cysts.....	4	0	4	0	10	
June 1	DE 1 Leaf....	Flagellates	2	0	2	0	8	June 24
June 19	DE 4 Leaf....	None	6	0	6	0	2	July 17
	DE 7 Leaf....	None	6	0	6	0	2	
June 20	DE 7 Leaf....	Amoebae, flagellates, cysts	5	0	5	0	4	July 17
July 13	DE 4 Leaf....	None	5	0	5	0	5	July 27
	DE 7 Leaf....	Amoebae, cysts	5	0	5	0	5	
		Total.....	56	0	56	0	71	

tion, it is essential to note these various factors in evaluating the experimental data given in tables II, III, and IV. Furthermore, in view of the fact that the stage of the organism might be a factor in infection, it is significant that it was possible to carry out inoculations with all stages of the life history of the organism reported by JONES save that of the plasmodium. Even here, however, some relevant data were obtained in filtration-inoculation experiments. All of the inoculations were made with mixed cultures save one set made on July 30. This was made with a pure culture of cysts and flagel-

lates in Knop's solution obtained from a single plasmodium which had been isolated and transferred with a capillary pipette. Inoculations were made by means of needles with a wrapping of cotton. In the later experiments each plant was inoculated with a separate sterilized needle. The needle was placed in the inoculum until the cotton had become thoroughly soaked, and the leaves were then punctured and scarified. The controls were punctured and scarified with sterile needles. These experiments are reported in tables II and III.

In table II are grouped the inoculation experiments in which cultures of diseased tissues were used, and in which the disease was

TABLE IV

INOCULATIONS WITH CULTURES FROM HEALTHY PLANTS

DATE OF INOCULATION	SOURCE OF CULTURE	PROTOZOAN IN CULTURE	INOCULATED PLANTS			CONTROLS		DATE OF CONCLUSION OF EXPERIMENT
			Total	Diseased	Healthy	Diseased	Healthy	
June 18	HE 1	Amoebae, flagellates	6	0	6	0	4	July 17
	HE 2	Flagellates, cysts	6	0	6	0	4	
June 19	HE 1	Amoebae, flagellates	5	0	5	0	16	July 17
	HE 2	Flagellates, cysts	5	0	5	0	16	
July 13	HE 1	Gametes	5	0	5	0	5	July 27
	HF 1	Amoebae, flagellates	4	0	4	0	5	July 27
July 30	HE 3	Plasmodia, flagellates	10	0	10	0	10	August 14
		Total	41	0	41	0	60	

produced either in all or only some of the plants; while in table III are reported the experiments in which inoculation with cultures of the diseased tissues completely failed to produce the disease. Some of these cultures (tables II and III) did not show the organism at time of inoculation.

Out of the 214 control plants inoculated, only one developed mosaic; out of the 215 plants inoculated with cultures, 109 developed the disease; and of the 67 plants inoculated with cultures which at the time of inoculation did not show the organism (although they came from diseased plants), 28 developed mosaic.

In table IV are reported the results of inoculations with cultures obtained from healthy plants. *P. tabaci* was present in abundance

in these cultures. One of these (HE 3) was a pure culture of cysts and flagellates derived from a single plasmodium. Forty-one plants were inoculated and none developed the disease. Sixty controls were used and remained healthy.

On the supposition that this organism, if it were the causal agent of mosaic, might enter through the roots of plants, an experiment was conducted in which the cultures from diseased plants were poured on the soil of potted plants. In one lot the roots of the plant were cut, while in the other they were left intact. Out of 14 plants with cut roots, 12 developed the disease, while out of 43 plants with the roots intact, only one developed it. Twelve plants were used as controls and all remained healthy.

III. Filtration and inoculation experiments

In view of the enormous mass of evidence that the causative agent of mosaic passes through diatomaceous and porcelain filters, it seems reasonable to suppose that if *P. tabaci* is the causative agent it must have some filterable stage. In the experiments of this section infective tobacco juice was filtered, the filtrate again tested for infectivity, then allowed to stand for several weeks to see whether *P. tabaci* appeared; and finally, as *P. tabaci* did not develop in any of the filtrates, these were tested for their suitability as culture media by inoculation with a known strain of *P. tabaci*. A few filtration experiments were carried out also with cultures of *P. tabaci*. In these experiments the work was so divided that the grinding of diseased tissues for juice was done by one person, the filtration by another, and the inoculation of plants by the third. This was to avoid all possibility of transmission of the virus by the person carrying out inoculations. Some of the filtrates were filtered into sterile flasks containing sterilized leaf tissues which might provide a suitable food medium for the organism, in case it should pass through the filters, and others were filtered into flasks containing only cover glasses. In one of the experiments (14 J and 15 J) the juice of each diseased plant was filtered through a separate filter, but the filtrates were then divided into several flasks. In the filtration work, Mandler, Berkefeld, and Pasteur-Chamberland filter candles were used. In each case the entire apparatus was set up complete, wrapped in

paper, and autoclaved for 30 minutes at 15 pounds pressure. When the diatomaceous candles were used an agar seal was deposited around the bottom of the candle just before use, in order to avoid leakage. The Pasteur-Chamberland candles were so arranged that the material was sucked into the candle and passed through a section of pressure tubing into a flask. Before filtration the tissue extract or the cultures were heavily inoculated with *Bacillus prodigiosus*. The filtrates were tested for *B. prodigiosus* 12 hours after filtration and again (24 hours later) when part of the filtrate was removed with sterile pipettes for inoculation purposes. Relevant data in regard to filtration conditions, such as pressures, time, P_H, etc., are given in table V. In no case did *B. prodigiosus* pass through the two Pasteur-Chamberland filters that were used. About half of the diatomaceous (especially the Mandler) candles leaked *B. prodigiosus* and were therefore discarded. None of the experiments with faulty filters are included in the table.

The filtration experiments with tissue extracts can conveniently be considered in two lots. In the first lot (table V, 4–13) the filtrates were free from *B. prodigiosus* 24 hours after filtration. In the operation of removing material for inoculation, however, they were opened several times in a very dusty room, and all later became infected with common air contaminants. As many stages of *P. tabaci* feed upon bacteria, it was felt that such contaminated filtrates would be particularly conclusive if the mycetozoan did not develop in the filtrates. An examination of the data in table V shows that although these filtrates were highly efficacious in producing mosaic, *P. tabaci* developed in none of them. This is true although the filtrates contained what we consider suitable food for the mycetozoan, and in spite of the fact that later inoculations of the filtrates with *P. tabaci* showed conclusively that the mycetozoan could live and grow in such a medium.

In the second lot of filtrations (14 J and 15 J) the filtrates were opened in a dust-proof chamber, and hence showed less (5 out of 14) contamination from the air. The contaminated cultures were discarded. The data gained from this experiment (table V) again show that the filtrates were infective to tobacco plants, but in no case did *P. tabaci* appear in them. Here, again, later inoculation of the fil-

trates with *P. tabaci* showed that the mycetozoan could live and grow in them.

The third series of filtrations, inoculations, and cultures was carried out with cultures of *P. tabaci* (table V). In filtrations 4 C and 9 C cultures containing plasmodia were used. The filtrates of these cultures did not develop the organism, although subsequent inoculations with a culture of *P. tabaci* proved that the filtrates were a suitable medium for growth of the mycetozoan. The filtrates were

TABLE V

FILTRATION, INOCULATION, AND CULTURE EXPERIMENTS WITH JUICES OF DISEASED PLANTS, AND CULTURES OF P. TABACI

Material*	Filter*	Pressure in cm. of Hg.	Time in minutes	P_H of solution	Inoculations of plants						Examination of filtrate		Examination of filtrate 7 days after addition of culture of mycetozoan
					Date	Date of close of observations	Controls		With filtrates		July 30	August 7	
							Total	Diseased	Total	Diseased			
4–C..	B	63	5	8.0	7/10	7/17	5	0	7	0	0	0	Amoebae, cysts; Bac.
5–J..	B	63	20	6.6	7/10	7/17	5	0	7	7	Bac.	Bac.	Bacteria
7–J..	M	67	120	6.4	7/12	7/19	5	0	7	5	Bac.	Bac.; cysts	Cysts; Bac.
9–C..	B	65	30	8.0	7/14	8/7	5	0	7	0	Bac.	Bac.	Amoebae, cysts; Bac.
11–J..	P	65	20	6.4	7/16	7/23	5	0	4	4	Bac.	Bac.	Flagellates; Bac.
12–C..	B	70	15	6.6	7/16	7/22	4	0	4	4	Bac.	Culture lost	Culture lost
13–J..	P	65	20	6.4	7/16	7/22	4	0	4	4	Bac.	Culture lost	Culture lost
14–J..	P	64	25	6.4	8/3	8/20	10	0	10	10		August 14	.....
a..	...											Bac.	Culture discarded August 14
b..	...											Bac.	Culture discarded August 14
c..	...											Fungus	Flagellates; Bac.
d..	...											0	Flagellates; Bac.
e..	...											0	Flagellates; Bac.
f..	...											0	Flagellates; Bac.
g..	...											0	Flagellates, cysts; Bac.
15–J..	P	64	25	6.4	8/3	8/20	10	0	10	10		August 14	.....
a..	...											Bac.	Culture discarded August 14
b..	...											Bac.	No stage of mycetozoan; Bac.
c..	...											0	No stage of mycetozoan; Bac.
d..	...											0	Cysts; Bac.
e..	...											0	Flagellates, cysts; Bac.
f..												0	Flagellates, cysts; Bac.
g..	...											0	Flagellates, cysts; Bac.

* C, culture of mycetozoan from diseased plants; J, juice extracted from diseased plants: 5 J, 7 J, 11 J, and 13 J, separate lots of juice; 14 Ja–g and 15 Ja–g, portions of two separate lots of juice; B, Berkefeld filter; M, Mandler filter; P, Pasteur-Chamberland filter.

not infectious. Unfortunately, however, it developed later that we do not have, as was thought when these cultures were filtered, any data relative to the infectivity of the cultures before filtration; consequently these experiments merely prove that the plasmodial stage of the organism did not pass the filters. 12 C was a culture from a diseased plant (DE 5, tables I and II). This culture had proved consistently infective during months when inoculated into plants. It never developed recognizable stages of the mycetozoan, however, but at one time it contained bodies which JONES thought might be a gametic stage. The filtrate was infectious and the organisms did not develop in the filtrate, although it proved to be a suitable medium when inoculated with a culture of *P. tabaci*. These data again indicate that the stages of the organism tested were not filterable.

Discussion

The culture experiments listed under I, although inconclusive in regard to the possible etiological rôle of *P. tabaci* in mosaic disease, bring out several facts of importance in regard to the distribution of the mycetozoan. They conclusively demonstrate that some stage of the organism, probably the cysts, are on the surface of many healthy and diseased plants. After a partial sterilization of the surface of the plants, the organism was found much more frequently in (or on) diseased than healthy plants. It would seem that this may be explained in one of three ways:

1. Diseased plants may present a rougher surface, and hence resistant stages of *P. tabaci* could more easily escape contact with mercuric chloride than in the case of healthy plants. If this possibility be the true one, *P. tabaci* would be considered only as an accidental contaminant of the plant's surface.

2. Diseased plants may present more breaks in the epidermis than healthy ones. It is possible that *P. tabaci* penetrates such breaks and lives within the parenchyma. If this be true, *P. tabaci* would probably be a secondary invader without any significant part in the production of this disease. Such an explanation has much in its favor, as it agrees with the rest of the experiments which indicate that the mycetozoan is not the causative agent, and it agrees with JONES' (*loc. cit.*) contention that the plasmodium of *P.*

tabaci can be found in the tissues of the leaf. It also is not out of harmony with the fact that some healthy leaves, even after washing in mercuric chloride, show the organism, as these may have been slightly damaged in other ways than by mosaic disease.

3. There is the possibility that *P. tabaci* is a frequent parasite of tobacco plants, and that it is the primary invader which later allows the entrance of the virus of tobacco mosaic. At present there seems little to commend this possibility.

The inoculation experiments listed under II indicate beyond reasonable doubt that, for the stages tested, *P. tabaci* is not the causative agent of tobacco mosaic, because cultures of the protozoan were effective in producing mosaic only when originally obtained from diseased plants, never when obtained from healthy ones. Furthermore, some cultures made from diseased plants and never showing the organism produced the disease. These results are just what would be expected if the disease is produced by an unknown virus, and *P. tabaci* is a common contaminant of many plants.

The filtration and inoculation experiments listed under III indicate further that *P. tabaci* is not the causal agent of mosaic. All the filtered juices from diseased plants produced the disease promptly, but none developed the organism in culture, in spite of the fact that later inoculation of the filtered juice with *P. tabaci* showed that it was a suitable medium for the growth of the mycetozoan. Similar filtration and cultural experiments with cultures of the plasmodial stage organism proved that it does not pass the filters used in the stage which JONES reported as occurring in diseased tissues.

Conclusions

1. *Plasmodiophora tabaci* can be cultivated from both healthy plants and those affected with mosaic, provided they are not washed in an antiseptic solution such as mercuric chloride. If they are washed in mercuric chloride *P. tabaci* can frequently be obtained from diseased but only rarely from healthy plants.

2. Inoculation of tobacco plants with cultures containing various stages of *P. tabaci* is followed by mosaic only when the cultures are derived from diseased plants, in which case a concomitant mosaic virus could be present and carried by dilution.

3. Cultures prepared from diseased leaves are sometimes negative for *P. tabaci* and still efficacious in the production of mosaic.

4. Filtrates from diseased plants are infective, but do not show *P. tabaci* after standing various lengths of time. Furthermore, such filtrates are suitable culture media for *P. tabaci*, as was demonstrated at the end of each experiment.

5. *P. tabaci* in the plasmodial stage does not pass the filters.

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TEMPERATURE AND RESPIRATION OF RIPENING BANANAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 361

ALBERT JACKSON OLNEY

(WITH ONE FIGURE)

Introduction

The appearance of the banana as an important fruit on the American market is relatively recent, although it has been cultivated and used from earliest times. The first shipment to the United States (**25**) was made in 1804, and another in 1830, but it was not until 1866 that bananas were imported regularly, and then only in modest quantities to seaboard towns. During the last twenty-five years the banana trade has been extended throughout the United States until now it represents an important industry.

Bananas must be picked while still green, in order to withstand the long journey from the tropical countries to the markets. A week to ten days are required for transportation to points in the United States. If ripening begins in transit the fruit deteriorates before it reaches its destination, and results in heavy losses. The problem of chief concern, therefore, is that of finding improved methods of retarding the ripening in transit and storage so that the fruit may reach the market in a condition of high quality and attractive appearance. The degree of maturity when harvested is determined by the growers in a purely empirical way, the degree of maturity when picked depending on the distance to be shipped. Those going to Europe are harvested somewhat greener than those for the United States, and the greener lots are said to be poorer in quality than those picked when more mature (**13**). Shipments are made in specially constructed steamships providing reduced temperatures by ventilation or refrigeration. Naturally ventilated steamships are commonly used for shipments from Central America or Jamaica to the Gulf ports. Distribution is then made to the central portions of the country in refrigerator cars. Refrigeration shipments are held as near 16° C. as possible.

After arriving at their destination the bunches are hung in curing rooms at 17°–20° C., providing 82–86 per cent humidity. The trade considers 15° C. the lowest safe temperature for green bananas, and 14° C. as the minimum for ripe ones. Chilling is said to occur at lower temperatures, injuring the quality and preventing the development of attractive appearance. Cargoes sometimes arrive at the ports too ripe for general distribution, while other shipments apparently handled in the same manner arrive in good condition. The causes for such occurrences are not well understood by importers. There is a general belief that a few ripe or ripening bananas among a lot of green ones greatly accelerate the ripening of the cargo.

The first noticeable development of the green fruit is the gradual change of color, accompanied by a softening of the pulp, due to the hydrolysis of pectose to pectin (**17**). The change from green to yellow takes place in 3–5 days at curing temperatures after arriving at their destination. Two or three days after the bright yellow stage, brown specks develop through the yellow, and the pulp reaches its highest flavor, aroma, and quality. This stage is short; the pulp soon becomes too soft, loses flavor, and begins to decay. When green the peel is filled with a white viscous fluid and the pulp is acid, bitter, and astringent. GERBER (**14**) cites BOUSSINGAULT and CORENWINDER (**9, 10**) as attributing the acidity of bananas to malic acid, but he was able to show only the presence of citric acid. As the ripening continues the peel becomes dry and leathery, and the pulp increases in sweetness until the fully ripe stage is reached.

A number of investigators have studied various phases of the ripening process of bananas. The Jamaica Department of Agriculture (**27**) investigated the gases given off by oranges and bananas. The carbon dioxide liberated was thought to retard the ripening, but unknown gases or emanations which they thought were given off by the oranges were believed to induce premature ripening. The practical deduction was drawn that separate storage was desirable for the two sorts of fruit during sea transportation. The chemical changes taking place in ripening bananas have been reported by GORE (**16**), MARCANO and MUNTZ (**21**), RICCIARDI (**24**), DOHERTY (**12**), COLBY (**8**), BALLAND (**4**), ATWATER and BRYANT (**1**), CHACE, TOLMAN and MUNSON (**7**), PRINSEN GEERLIGS (**22**), YOSHIMURA

(**28**), REICH (**23**), TALLARICO (**26**), and BAILEY (**23**). Most of these analyses were made on green and ripe fruit. GORE (**16**) makes a sweeping criticism of the early analyses because their results were invariably expressed in terms of the percentage of the pulp when analyzed, so that the data are on a constantly shifting basis, since the peel continually loses weight while the pulp increases in weight during ripening.

LANGWORTHY and MILNER (**20**) studied the ripening of bananas in a respiratory calorimeter. They found that the volume of oxygen consumed was practically equal to the volume of carbon dioxide liberated. Their determinations show from the thermal quotient and carbon dioxide produced that the respiration was due entirely to the combustion of carbohydrates.

Materials and methods

The bananas used for the following experiments were obtained from the United Fruit Company through the kindness of Mr. J. W. LEATHERS, to whom thanks are gratefully accorded. Green bananas were obtained as soon as possible after arrival in Chicago. Two lots were received from Honduras, one ventilated and one refrigerated, and a ventilated and a refrigerated lot from Jamaica.

JOHNSTONE'S modification of Magness' respiration apparatus (**19**) was used to determine the amount of oxygen consumed and carbon dioxide liberated during the ripening. The apparatus was maintained at 20° C. in a Freas water bath. One hour was allowed for adjustments of temperature of the apparatus to that of the bath at the beginning of each experiment, and one-half hour between the daily runs when fresh quantities of NaOH were inserted for absorption of carbon dioxide. A Beckman thermometer was used to check the uniformity of the temperature of the bath. The temperature variation ranged from 0.05° to 0.20° C. The oxygen absorbed by the bananas was considered equal to the volume of water drawn into the cylinder. The amount of carbon dioxide liberated by the bananas was determined by titrating the NaOH, using the double indicator method (**5**). The individual bananas were cut apart and weighed before being put into the respiratory chamber. Later weighings were made at the end of each day's run. Four refrigerators operated at

2°, 7°, 12° and 17° C. were used for determining the effect of low temperatures on ripening.

Results

The results obtained from respiration studies are given in tables I, II, III, and IV. At the end of each experiment evidences of initial decay were usually apparent, although the pulp was in good condition. The maturity of the different lots was not uniform when re-

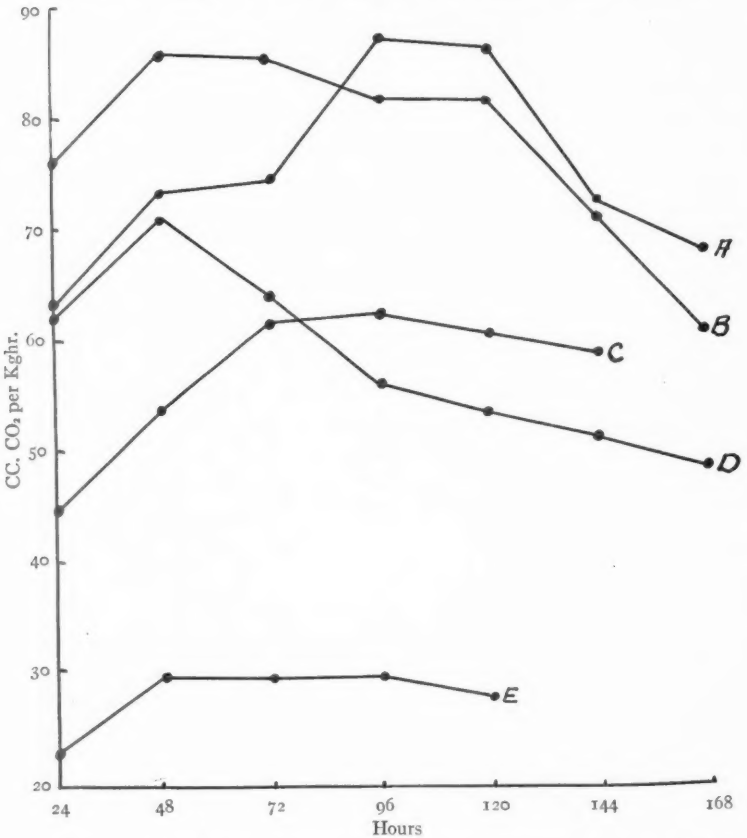

FIG. 1.—*A*, Honduras refrigerated 20°; *B*, Jamaica refrigerated 20°; *C*, Jamaica ventilated 20°; *D*, Honduras ventilated 20°; *E*, Jamaica refrigerated 12° C.

ceived, even when account is made for the method of cooling in shipment. The Jamaica ventilated lot appeared most mature, while the Jamaica refrigerated and the Honduras ventilated were similar in apparent maturity, and the Honduras refrigerated was greener than the others and not as ripe at the end of the experiment. Table V shows the daily loss in weight and production of carbon dioxide at 12° C. Fig. 1 indicates the nature of the respiration of the different lots at 20° C. and at 12° C. Table VI shows the effects of low temperatures on ripening.

The Jamaica ventilated bananas (table I) ripened very rapidly, and by the end of six days were becoming soft, showing initial decay,

TABLE I

JAMAICA VENTILATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PERCENTAGE LOSS IN WEIGHT	KGMHR	CALORIES PER KGMHR	cc. CO_2 PER KGMHR	cc. O_2 PER KGMHR	CO_2/O_2 PER KGMHR	REMARKS
March 14, 23 hours..	325.0	0.06	7.475	0.22	44.94	44.151	1.01	Slightly yellow
March 15, 25 hours..	324.8	0.18	8.120	0.27	53.71	49.261	1.09	Mostly yellow
March 16, 23.5 hours	324.2	0.27	7.618	0.31	61.57	55.131	1.11	Yellow
March 17, 23.5 hours	323.3	0.06	7.597	0.32	62.97	53.311	1.18	Yellow
March 18, 23.5 hours	323.1	0.18	7.593	0.31	60.50	50.041	1.20	Brown
March 19, 23.5 hours	322.5	0.28	7.578	0.30	58.80	46.221	1.24	Very ripe

and were too ripe for handling on the market; however, the pulp was sound and had a rich aromatic flavor. The fruit inclosed in the respiratory chamber seemed to decay more rapidly than samples of the same lot stored in the laboratory, due to higher humidity. The loss in weight during this experiment was very low, about 60 per cent being due to liberation of carbon dioxide. The kilogram hours were determined by multiplying the kilograms of bananas by the number of hours in the run. The calories of heat produced were calculated from LANGWORTHY and MILNER'S (**20**) determinations, showing that 2.58 calories of heat are produced for each gram of carbon dioxide liberated. The volume of carbon dioxide given off and oxygen consumed were nearly equal in the daily runs, the respiratory quotient gradually increasing from 1.01 for the first run

to 1.24 at the end. There was a gradual increase in the volume of carbon dioxide produced until the bananas were fully yellow, followed by a slight decline.

The Jamaica refrigerated lot appeared green when received but ripened rapidly. The daily loss in weight was fairly uniform, and averaged about 0.4 per cent of the weight of the fruit per day, of

TABLE II

JAMAICA REFRIGERATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PER-CENT-AGE LOSS IN WEIGHT	KGMHR	CALOR-IES PER KGMHR	CC. CO_2 PER KGMHR	CC. O_2 PER KGMHR	CO_2/O_2 PER KGMHR	REMARKS
March 28, 24 hours..	770.1	0.53	18.48	0.38	75.98	77.65	0.97	Green
March 29, 23 hours..	766.0	0.45	17.61	0.44	86.42	83.19	1.03	Yellowing
March 30, 23.5 hours	762.5	0.43	17.90	0.43	85.13	76.81	1.10	Mostly yellow
March 31, 23 hours..	759.2	0.41	18.20	0.42	82.47	76.37	1.07	Yellow
April 1, 24 hours....	756.1	0.41	18.40	0.42	83.72	74.97	1.11	Yellow
April 2, 23 hours....	752.9	0.42	17.30	0.36	70.48	60.11	1.17	Brown
April 3, 23 hours....	750.3	0.34	17.20	0.30	60.00	59.30	1.01	Very ripe

TABLE III

HONDURAS VENTILATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PER-CENT-AGE LOSS IN WEIGHT	KGMHR	CALOR-IES PER KGMHR	CC. CO_2 PER KGMHR	CC. O_2 PER KGMHR	CO_2/O_2 PER KGMHR	REMARKS
April 11, 23 hours...	688.4	0.40	15.83	0.32	63.21	64.10	0.99	Light green
April 12, 23 hours...	685.6	0.42	15.76	0.36	71.34	62.78	1.13	Yellowing
April 13, 23 hours...	682.7	0.38	15.70	0.33	64.45	62.41	1.08	Mostly yellow
April 14, 23 hours...	680.1	0.35	15.64	0.29	56.52	53.06	1.06	Yellow
April 15, 23 hours...	677.7	0.37	15.58	0.27	54.27	49.08	1.10	Yellow
April 16, 23 hours...	675.2	0.34	15.53	0.26	52.48	49.58	1.05	Browning
April 17, 23 hours...	672.9	0.33	15.47	0.25	48.82	47.86	1.02	Very ripe

which 31 per cent was due to loss of carbon dioxide. The volume of carbon dioxide produced was considerably greater per kilogram per hour than that produced by the Jamaica ventilated lot. The greatest production of carbon dioxide occurred on the second day, followed by a gradual decline to the end of the yellow stage, and then a rapid falling off. The respiratory quotient was slightly more than unity except for the first day.

The Honduras ventilated bananas ripened at approximately the same rate as the Jamaica refrigerated, and the daily loss in weight was similar in amount. The largest production of carbon dioxide occurred on the second day and gradually declined to the end of the experiment. The respiratory quotient was slightly above unity in most cases.

TABLE IV

HONDURAS REFRIGERATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PERCENTAGE LOSS IN WEIGHT	KGMHR	CALORIES PER KGMHR	CC. CO_2 PER KGMHR	CC. O_2 PER KGMHR	CO_2/O_2 PER KGMHR	REMARKS
April 18, 23.5 hours.	154.5	0.50	3.630	0.32	62.80	63.35	0.99	Green
April 19, 23.5 hours.	153.7	0.50	3.612	0.37	72.65	66.44	1.08	Slightly yellow
April 20, 23.5 hours.	152.9	0.39	3.593	0.38	74.59	73.75	1.01	Yellowing
April 21, 23.5 hours.	152.3	0.46	3.579	0.44	87.79	89.41	0.99	Yellow
April 22, 23.5 hours.	151.6	0.39	3.562	0.43	85.29	84.22	1.01	Yellow
April 23, 23.5 hours.	151.0	0.39	3.548	0.37	72.71	66.38	1.09	Slightly brown
April 24, 23.5 hours.	150.4	0.33	3.534	0.34	67.68	67.91	0.99	Ripe

TABLE V

JAMAICA REFRIGERATED; RESPIRATION AT 12° C.

DATE AND TIME OF RUN	WEIGHT	PERCENTAGE LOSS IN WEIGHT	KGMHR	CC. CO_2 PER KGMHR	REMARKS
March 28, 24 hours.....	585.0	0.29	14.04	22.27	Green
March 29, 23 hours.....	583.3	0.25	13.42	29.96	No change
March 30, 22 hours.....	581.8	0.22	13.80	29.13	No change
March 31, 22 hours.....	580.5	0.24	12.77	29.73	No change
April 1, 23 hours.....	579.0	0.34	13.31	26.85	Slight change

. The Honduras refrigerated bananas were less mature when received than any of the other lots. The loss in weight for the first day was 0.5 per cent of the weight of the fruit, and declined to 0.3 per cent for the last day of the experiment. About 25 per cent of this loss was due to liberation of carbon dioxide and the remainder to transpiration. The volume of carbon dioxide was relatively high per kilogram per hour, and higher than the Honduras ventilated, the maximum occurring on the fourth day. The respiratory quotient was more nearly unity than any of the other lots.

The respiration of the Jamaica refrigerated lot at 12° C. is shown in table V. The daily loss in weight was reduced to nearly one-half and the volume of carbon dioxide produced to about one-third that at 20° C.

The effect of low temperatures on the ripening of bananas is shown in table VI. The lots held at 2°, 7°, and a part of those at 12° C. were transferred April 5 to the 17° refrigerator, and the remainder of those held at 12° were continued at that temperature. The lot held at 17° ripened in six days, while those held at the lower

TABLE VI

EFFECT OF LOW TEMPERATURE ON RIPENING (PUT IN REFRIGERATORS MARCH 28)

DATE OF EXAMINATION	2° C	7° C	12° C	17° C
March 30...	Green	Green	Green	Quite yellow
April 1.....	Slightly darker	No change	No change	Yellow
April 3.....	Same	Same	Same	Ripe (removed)
April 5.....	Same	Same	Slightly yellow	
	Put at 17°	Put at 17°	Part put at 17° and part continued at 12°	
April 7.....	Slight	Slight	Little change	
April 7–14..	Gradual change to a dull yellowish brown with black and dark brown areas; no bright yellow stage; pulp became soft, poor quality, lacking aroma; no marked differences in the three lots			Gradual change to yellowish green with black areas; remained hard; not eatable

temperatures showed little change during this time, and when transferred to ripening temperature failed to ripen normally. After eleven days they had developed a dull unattractive appearance and lacked quality and aroma. Those held continuously at 12° C. were still hard and green at the end of the experiment, although they were beginning to show signs of decay.

Discussion

The changes taking place during ripening of bananas are rapid, and the fully ripe period is very short, usually lasting about two or three days at ordinary temperatures. The rate of respiration at 20° C. was found to increase rapidly during the first two or three days and then gradually declined. The refrigerated lots, tables II and IV,

gave a high respiration rate followed by a rather sharp decline; while the ventilated lots, tables I and III, did not respire at so high a rate and declined more gradually. This difference in respiratory activity may have been due to an accumulation of sugars at the low temperatures, so that a more rapid respiration occurred when brought into ripening temperatures. The volumes of carbon dioxide liberated and oxygen absorbed were practically equal, as shown in the tables. In the combustion of carbohydrates the volume of carbon dioxide produced is exactly the same as that of oxygen consumed; that is, the respiratory quotient is 1.00. It appears therefore that the oxidation of carbohydrates is the principal process involved. This is also confirmed by inspection of analyses of the banana (**16**), which show that carbohydrates and water comprise about 96 per cent of the whole fruit, with less than 1 per cent of fats or proteins. Further to the point, LANGWORTHY and MILNER determined that the thermal quotient for normal ripening bananas indicates that the carbon dioxide produced is due solely to the complete combustion of carbohydrates. They found that 2.58 calories of heat are produced for each gram of carbon dioxide eliminated. Calculations on this basis show that approximately 0.3 calorie is produced per kilogram per hour during ripening at 20° C. This means the production of 10–15 calories of heat per hour from a single bunch of bananas at maximum respiration, and shows the possibility of a considerable rise in temperature in the ship or car during transportation. This may be an important factor in causing the occurrence of occasional shipments which become over-ripe in transit. It is important, therefore, not only that the surrounding temperature should be carefully regulated, but that proper ventilation should be provided to carry away the heat formed by the bananas themselves. It has been suggested that the heat liberated by the bananas during ripening may be due in part to bacterial action, but bacteriological studies by BAILEY (**3**) indicate that the inner part of the pulp is practically sterile, while the inner portion of the peel is sparsely inhabited by bacteria which he found were practically inactive until the fruit became ripe.

The loss in weight during ripening was relatively low, due to the highly cutinized surface of the peel and the high humidity maintained throughout the experiments. About one-fourth of the loss

was due to escape of carbon dioxide, and the remainder to transpiration of water. During ripening the pulp increases considerably in water content, due partly to absorption of water from the peel because of increased osmotic pressure as starch is changed to sugar, and partly from the combustion of glucose according to the equation $C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O$. The amount of carbon dioxide liberated at 12° was reduced approximately to one-third that at 20° C. The volume of oxygen absorbed at this temperature was not determined, and consequently the respiratory quotient cannot be shown, but the efficacy of reduced temperatures in retarding ripening is clearly indicated.

On March 28, samples of green bananas were put in refrigerators at 2°, 7°, 12°, and 17° C. Those held at 17° were fully ripened in six days, while those held at the lower temperatures were still very green. After eight days those held at 2° and 7°, and a part of those at 12° were put in the 17° refrigerator and held there for eleven days. It was soon strikingly apparent that the pre-refrigeration at 2°, 7°, and 12° had made a decided change in the character of ripening when placed at 17° C. All lots ripened slowly. The change in color was very gradual and the bright clear yellow failed to develop. At the end of the experiment the appearance of all lots was dull and unattractive, yellowish brown in color, spotted with blackened areas, and showing evidences of initial decay. The pulp had softened and appeared to be in good condition. The quality was almost as good as some often obtained on the market, but was noticeably flat and decidedly lacking in the aroma so chracteristic of properly ripened bananas. These results are in agreement with the earlier work of GERBER (**14**). This indicates that a temperature of 12° or lower not only retards ripening but permanently affects the fruit, so that normal ripening does not occur when brought into ripening temperatures.

Summary

1. At 20° C. green bananas ripen rapidly, becoming fully ripe in 5–7 days after arrival in Chicago.

2. The respiratory quotient is approximately unity, indicating that the oxidation consists solely of the combustion of carbohydrates.

3. The rate of respiration increases rapidly at the beginning of ripening and falls off gradually.

4. Refrigerated bananas had a higher rate of respiration than those naturally ventilated, and declined more rapidly as they became ripe.

5. Respiration at 12° was reduced to about one-third that at 20° C.

6. The daily loss in weight was relatively low under the conditions of these experiments.

7. Approximately 0.3 calorie of heat is produced per kilogram per hour by ripening bananas at 20° C.

8. Refrigeration at 12° C. or lower not only retarded ripening in storage, but permanently prevented normal ripening when transferred later to a ripening temperature. The low temperatures prevented the development of bright yellow color, high quality, and aroma.

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STRUCTURE OF PENNSYLVANIAN PLANTS FROM ILLINOIS. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 362

J. HOBART HOSKINS

(WITH PLATES XXIII, XXIV)

Through the courtesy and interest of Mr. JULIUS HEGLER, of the Hegler Zinc Company, Danville, Illinois, the model coal mine of that company has been open for collecting, with the result that a number of coal balls have been obtained by Professor A. C. NOÉ. Some of these petrifactions were kindly turned over to the writer, and a series of sections from one of these coal balls constitutes the material basis of this paper.

The Hegler coal mine produces coal from the Danville vein, known locally as no. 7 coal, and charted in that formation of the Pennsylvanian period known generally in Pennsylvania as the Conemaugh, and in Illinois as the McLeansboro. Unpublished investigations of this coal seam by paleobotanists and stratigraphers indicate that this vein may ultimately be given a higher position than that by which it is now known.

The Danville vein at the Hegler mine has a thickness of about 5 ft. 4 in. Below the coal is the usual fire clay, while above is a 7 ft. layer of black shale with flakes of pyrite, in which occur numerous concretions. The petrifactions occur in groups or pockets in the upper 2 ft. of the coal. They are calcified, with pyrite occurring in varying amounts.

From one of these coal balls was obtained a series of sections which gives the structure of the ultimate rachis of a fernlike frond with pinnules bearing fructifications. The pinnule is of the *Pecopteris* type. The midvein is prominent and extends almost to the tip of the pinnule. The lateral veins, usually seven to nine on either side of the midvein, are given off at a wide angle and are simple. The pinnules are 6–7 mm. long and about 3.3 mm. in width, and are furled so that their edges approach the lower side of the midvein, forming a semi-chamber within which the fructifications are attached.

Sporangia occur in small groups or sori. These are arranged in a single series on each side of the median vein on the lower side of the pinnule. There is not a wide variation in the number of sporangia found in the different sori. With the exception of one group of six sporangia, the number in each sorus is either four or five. Groups of four are about twice as numerous as groups of five. Adjacent sori may vary in number.

The sporangia are found only in the mature condition with dehiscence either already completed, or with the spores completely separated apparently ready to be dispersed. The sporangia of each sorus are but slightly connected laterally at their bases, and are attached to a common pedicel to the lower surface of the pinnule. The sporangia in cross-section are roughly cylindrical, with the adjacent sides slightly flattened. The sporangia swell slightly below their points of attachment, so that their maximum diameters occur a little above their bases. The outline of the entire sorus in cross-section is somewhat star-shaped, with extremely obtuse points. In longitudinal section the sporangial cavity is ovoid in shape, with the longer axis at right angles to the pinnule. The distal ends of the sporangia are considerably prolonged beyond the sporangial cavity, to form bluntly acute apices by the elongation of the sporangial wall cells of this region on the sporangium.

The sporangia average 0.28 mm. in diameter and about 0.70 mm. in length. Spores are commonly found in large numbers within the sporangia, and average 0.017 mm. in diameter. Neither marking of the spore coat nor spore content can definitely be distinguished. Dehiscence occurred through a vertical cleft extending from near the point of attachment of the sporangia to near the free end, on the inner face of the sporangia.

The sporangial wall varies greatly in thickness in different parts of the sporangium. In most sections it appears to be but one cell in thickness, but in favorable sections there are good indications of two additional layers of cells closely applied to the outer prominent wall cells. These inner layers, where they occur, consist of small thin walled cells, elongated parallel to the long axis of the sporangia.

The cells of the outermost sporangial wall layer differ considerably in size. Those on the outer free surface in cross-section are

approximately square, the outer tangential wall being slightly longer than the inner wall, and comparatively large with exceedingly thick cell walls. Many of the cells have a dark content. A longitudinal section through this surface shows that these cells have a length about twice their width, and that the outline of their cell walls gives a reticulated appearance.

The cells of the adjacent walls are much smaller and have their cell walls less thickened. The smallest cells are at the very innermost part of the sporangia, where the cleft for dehiscence occurs. In cross-section the cells of the laterally adjacent walls of the sporangia are rectangular, the radial width being about half the tangential width.

The maximum elongation of the sporangial wall cells is seen at the distal end, where they form a continuation of the sporangium into a bluntly acute apex for a considerable distance beyond the sporangial cavity. The distal ends usually are slightly concrescent toward a common center. Due partially to the fact that the extreme distal end is slightly curved, and partially to loss before petrifaction, longitudinal sections usually fail to show the extreme tip of the sporangium. These sections then give the appearance of a sporangium with a rather rounded free end, capped by a group of larger heavy walled cells which seem to extend for a distance up the free side toward the base of the sporangium. In reality, however, the apices of the sporangia are not rounded, but more or less acute, the free end being prolonged beyond the sporangial cavity.

The pinnules to which the sporangia are attached have in transverse section one or two rows of larger heavy walled cells beneath the upper epidermis, which in almost every instance is not preserved. Below is a region of thin walled cells more or less compactly arranged and rather circular in section. In some sections there seems to be a slight development of palisade tissue beneath the heavy walled tissue. No stomata have been identified positively.

The midvein of the pinnule consists of a small circular group of tracheids accompanied by a few non-lignified elements. There is not the U-shaped bundle which is characteristic of the ultimate rachis. The sporangia are supplied by lateral veins, a few spirally thickened elements deploying and ending in the upper portion of the pedicels which attach the sori to the pinnules.

The ultimate rachis bearing the pinnules has a vascular system in the form of a wide U. The xylem cells are small in caliber and rather uniform in size, although those at the free ends of the U are slightly smaller and in longitudinal section are found to be spirally thickened in place of scalariform, as are the others. Fig. 7 shows the vascular connection of the ultimate rachis and pinnule. A small group of tracheids is separated from a free point of the bundle of the rachis and almost immediately turns sharply outward into the pinnule forming the midvein, to which a small number of lateral veins are connected.

Surrounding the xylem of the bundle are three or four rows of thin walled cells, of about the same caliber as the xylem, and bounded externally by a very narrow region (scarcely a row, although certainly appearing so in some places) of very small and thin walled cells. In the section figured it is not demonstrated that these very small and thin walled cells completely encircle the tracheids, as they are not identified immediately within the U. Most transverse sections of the ultimate rachis, however, give the appearance of having this tissue entirely surrounding the vascular bundle.

Many of the large cells beneath the vascular bundle of the ultimate rachis show a dark content and were probably mucilage or tannin cells. A band of sclerenchyma extends from between the points of the U-shaped bundle to the epidermis between the places of attachment of the pinnules. Multicellular hairs are sparingly present on the lower side of the rachis and pinnules.

The following summary of the sporangial characteristics of this specimen may facilitate comparisons with other fernlike fructifications: Sori composed of four or five, rarely six sporangia, and attached by short pedicels to the lower surface of pinnules of the *Pecopteris* type in a single series on either side of the midvein. Pinnules about 6–7 mm. long and 3.3 mm. in width. Sporangia ellipsoidal, roughly circular in transverse section with adjacent sides slightly flattened, and attached laterally at their very bases. Distal ends extended into slightly concrescent bluntly acute apices by the elongation of the sporangial wall cells. Sporangia about 0.28 mm. in diameter and 0.7 mm. in length. Wall cells on free surface of the sporangium much larger and with heavier walls than those on

the adjacent walls. Dehiscence occurs through vertical cleft on the innermost wall of the sporangia. Spores numerous, averaging 0.017 mm. in diameter.

In his classification of fernlike fructifications, STUR (11) divided those which he considered to have Marattiaceous affinities into a number of subdivisions, based on the arrangement and structure of the sporangia. His suborder Asterotheceae has the following characterization:

> Sporangia ellipsoidea, apice plerumque libere subacumibata, dorso non rare in gibberum inflata, apicibusque conniventia ideoque connata, licet in sorum, synangium plus minus perfectum systentem, sessilem vel breviter pedunculatum, rotundum, stellatim congenta.

It would appear that the specimen just described belongs within STUR's suborder Asterotheceae. Within the Asterotheceae STUR placed four genera: *Asterotheca* Presl., *Scolecopteris* Zenker, *Renaultia* Stur (*Sturiella* Weiss), and *Diplozites* Goepp. (*Ptychocarpus* Weiss).

The structure of the fructification called *Ptychocarpus* (1) by WEISS (13) has been described by RENAULT (7). The fructifications are arranged in one or two series on either side of the median vein of pinnules of the *Pecopteris* type. A small number of sporangia, six to eight in the species described, *Ptychocarpus unita*, constitute a circular sorus. The sporangia are adherent to one another laterally and also to a common central receptacle, the whole sorus being imbedded in tissue which is continuous around the periphery of the sorus. Dehiscence probably occurred, according to RENAULT, through an apical pore. Very similar to this fructification is the one described by WATSON (12) under the name *Cyathotrachus altus*, which differs from *Ptychocarpus unita* in detail but not in general arrangement. These forms differ quite radically from the Illinois specimen, in the presence of the delicate tissue within which are imbedded the sporangia, in the lateral adhesion of the sporangia, and probably in the method of dehiscence.

The fructification known as *Asterotheca* (5) comprises a genus in which the sporangia, from three to eight, are grouped about a very short common receptacle, forming a circular sorus. *Asterotheca* is known to have been attached to pinnules of the *Pecopteris* type. The

sori are arranged in a single series on either side of the midvein and are sessile. The individual sporangium is ovoid in shape, with the free extremity flattened and ending in a small point. Dehiscence probably occurred by the springing apart of the sporangia when mature, the spores escaping through a vertical cleft on the inner wall of the sporangium. STUR used the generic name *Hawlia* for similar fructifications in which the sporangia were separate from one another to their bases, and places the genus in a separate suborder from the Asterotheceae. KIDSTON (**4**), however, places the Coal Measure form *Pecopteris Miltoni*, which STUR includes in *Hawlia*, in *Asterotheca*.

The general arrangement of *Asterotheca* is very similar to that of the Illinois specimen. It differs in certain important particulars. The sorus is not pediceled as is the one under consideration. The shape of the individual sporangium is usually different, that of *Asterotheca* being shorter and with the major axis usually parallel to the pinnule which supports it. It is especially different in the character of the free ends of the sporangium, which, in the form described is extended into a more or less acute apex by the elongation of the sporangial wall cells, and while there is a short apex in *Asterotheca*, does not approach the condition found in this specimen.

The genus *Scolecopteris* has the same general arrangement as *Asterotheca*. There is a relatively small number of sporangia grouped about a common receptacle, which in some species is extended to form a definite pedicel by means of which the sorus is attached to the pinnule, which is of the *Pecopteris* type. In other species the sorus is sessile. The sporangia are adherent laterally at their bases, but are free for most of their length. In the case of *Scolecopteris polymorpha*, the sporangia are laterally united for about one-third their length, which is possibly true of *S. elegans* as well. The distal ends of the sporangia are elongated into more or less acute apices, which are frequently somewhat concrescent. In some species there is a very long prolongation of the free ends, as in *S. polymorpha*.

With the species *Scolecopteris elegans*, the structure of which was described by STRASBURGER (**10**) in 1874, there are many points in common with the Illinois specimen. The sporangia are in groups of four and five, arranged in a single series on each side of the midvein of the pinnules of the *Pecopteris* type, the edges of which are strongly

revolute, partially inclosing the sporangia. The sporangia are distinctly pedunculate. The sporangia are concrescent toward the distal end, and, while coming to an acute point, do not have the long tapering free ends which are characteristic of *S. polymorpha*. The sporangium wall consists of more than one layer of cells, and is distinctly thicker on the outer free surface than on the radial adjacent walls. Dehiscence occurred by means of a longitudinal cleft in the sporangial wall on the inner face of the sporangium, where the wall cells are thinnest.

Scolecopteris elegans differs from the Illinois specimen chiefly in the matter of size, the sporangia of *S. elegans* measuring 0.4 mm. in diameter by 0.9 mm. in length, with spores 0.01 mm. in diameter, while the sporangia of the Illinois specimen are 0.28 mm. in diameter and about 0.7 mm. in length, with spores 0.017 mm. in diameter. Apparently the sporangia of *S. elegans* were adherent for a longer distance from their bases than in this specimen, as in no transverse section except through the very bases of the sporangia was it possible to identify a common sporangial wall such as figured by STRASBURGER for *S. elegans*.

It would seem that the differences between the Illinois specimen and *Scolecopteris*, especially *S. elegans*, are not sufficient to exclude the specimen from that genus, and it is therefore proposed to refer to the Illinois specimen as *Scolecopteris minor*, the specific name having reference to the less dimensions of the sporangia, in comparison with those species of *Scolecopteris* the structure of which has been described.

The fourth genus included in the Asterotheceae by STUR was his *Renaultia*. This form has been described originally by RENAULT (**6**) in 1883 under the name *Pecopteris intermedia*. Later that year STUR reviewed his work and included that fructification in his Asterotheceae under the name *Renaultia intermedia* Stur. The generic name *Renaultia*, however, had been employed slightly earlier by ZEILLER (**15**) for a form called *Hapalopteris* by STUR. Subsequently another generic name was proposed by WEISS (**14**), and the *Pecopteris intermedia* of RENAULT is known as *Sturiella intermedia* (Weiss).

The sporangia of *Sturiella intermedia* are arranged in groups of five and attached to the pinnule, which is of the *Pecopteris* type, by

a short pedicel in a single series on each side of the median vein. The sporangia were described as cylindrical, pyriform, with adjacent sides slightly flattened. The unique feature of the sporangium, however, was an apical annulus, which capped the distal end of the sporangium and extended on the free side for a distance toward the base of the sporangium. The pinnule which bore the sori had the vascular bundle in the shape of a crescent or wide U.

This description is interesting in view of the great similarity of this description with sections of *Scolecopteris minor* which have not passed exactly perpendicular through the sporangium. The longitudinal sections of *S. minor* frequently show a rather rounded apex, which, with the exception of the line for dehiscence, seems to be covered with a group of heavy walled cells which extend for a distance toward the base, dying out as it is approached. Similar sections were seen in a species of *Scolecopteris* in the British Museum. A transverse section through the prolongation of the sporangium at the distal end of *Scolecopteris minor* gives very similar views to those figured by RENAULT for *Pecopteris intermedia*, and which, in *S. minor*, consist of the heavy walled elongated cells of the sporangium wall with the cleft for dehiscence on the inner side.

Observations of RENAULT'S *Pecopteris intermedia* seem to indicate that some sporangia possessed more elongated apices than those figured, and that, in one instance at least, the vascular strand of the pinnule was composed of a small circular group of very small tracheids in place of the characteristic horseshoe-shaped bundle figured. While the hairs attached to the sporangia of RENAULT'S specimen are unique and not duplicated in known species of *Scolecopteris* with structure preserved, it would appear that there is perhaps less distinction between these genera than usually considered.

The natural affinities of *Scolecopteris* are not positively known, but although the relation of all fernlike fructifications has seriously been questioned as the result of the discovery that some at least were microsporangia of early seed plants, opinion regarding *Scolecopteris* in general is the same as that given by STRASBURGER in 1874 in regard to *Scolecopteris elegans:*

> ZENKER'S (16) *Scolecopteris elegans*, according to the formation of its sorus, certainly belongs to the Marattiaceae, and in fact, comes nearest the genus

Marattia in the form of the sporangia of which the sori are composed, while it approaches the genus *Kaulfussia* in the circular grouping of the sporangia, and finally resembles the genus *Angiopteris* in the fact that the sporangia become free in their upper part. In the mode of dehiscence of the individual sporangia, *Scolecopteris* agrees with all three genera mentioned, but the similarity with *Marattia* is again the most striking, to which it also bears the greatest resemblance in the structure of the sori.

To which SCOTT (**8**) adds, "So far as the pedicellate sori are concerned [and *S. minor* belongs here] the agreement is especially close with the sub-genus *Eupodium* of *Marattia*."

STRASBURGER, of course, wrote before the discovery of that extinct group of early seed plants, the Cycadofilicales, which has necessitated so much revision of thought in regard to fernlike impressions and fructifications. Whereas formerly it was naturally considered that a plant with fernlike foliage or sporangia belonged to the true ferns, it is now known that some species of practically every form genus of fernlike foliage belonged to seed bearing plants, and that the supposed fern sporangia were the microsporangia of seed plants. *Pecopteris* is no exception. Seeds have been found attached to at least one species, *P. Pluckenti* (**3**). Also *Crossotheca*, the bilocular sporangium of *Lyginopteris*, is listed by KIDSTON (**4**) as having been attached to three species of *Pecopteris*. It is true that no unilocular sporangium, such as *Scolecopteris*, is definitely known to have been the microsporangium of a seed plant, but according to SCOTT (**9**), "It is however probable that synangia such as those of *Telangium Scotti*, in which the sporangia are unilocular, may also have belonged to Lyginopterideae, and in that case an analogy with *Scolecopteris* would be evident; at present, however, our knowledge is too imperfect to justify further speculation."

More recently, however, unpublished researches by HALLE, according to a letter from Dr. SCOTT, gives excellent evidence that a new Pteridosperm bore seeds attached to the frond of a *Pecopteris* type of foliage similar to that on which has been found the *Asterotheca* type of sporangia.

On the other hand, the evidence that *Scolecopteris* is the fructification of a true fern has been strengthened by the anatomical work on *Psaronius*, which is generally considered to be the stem of an

arborescent fern with Marattiaceous affinities. GRAND'EURY (2) was convinced that the fronds of *Psaronius* were of the *Pecopteris* type, such as those which bear *Asterotheca* and *Scolecopteris*. This would indicate that these fructifications, in some instances at least, belonged to the true ferns. It is interesting to note in connection with the question of the relationship of *Pecopteris* fronds and fructifications with the stem of *Psaronius*, that closely associated with *Scolecopteris minor* in the petrifactions were numerous *Psaronius* rootlets, although it is well understood that close association in coal ball matetial is absolutely no evidence for previous organic connection.

In view of the close relationship between the *Asterotheca* and *Scolecopteris* type of fructification, however, conclusive evidence that the plant on which the *Asterotheca* type of sporangia occurs also bore seeds, as indicated by HALLE's investigations, would seem to warrant the assumption that *Scolecopteris* likewise is the male fructification of a member of the Cycadofilicales, conclusive evidence of which, however, must await further investigation.

Acknowledgment is due to Dr. A. C. NOÉ, whose generosity in regard to petrifactions and laboratory facilities made this paper possible, and to Dr. FREDDA REED for certain critical comments during the preparation of the manuscript.

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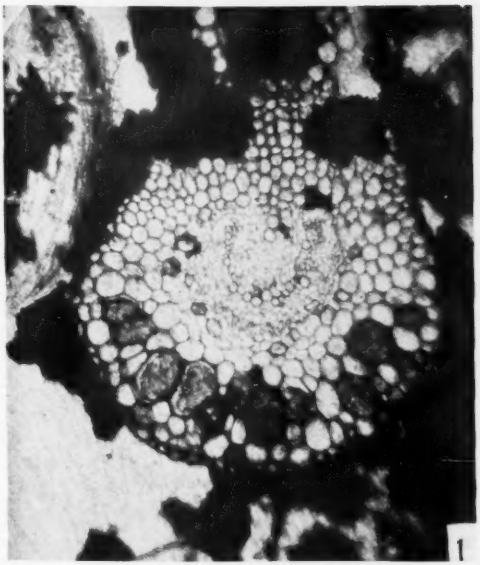
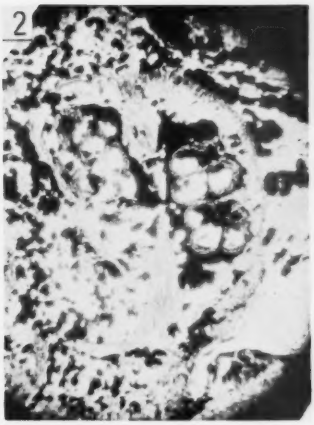
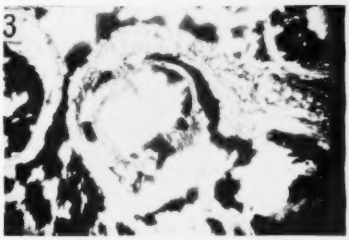
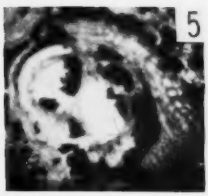
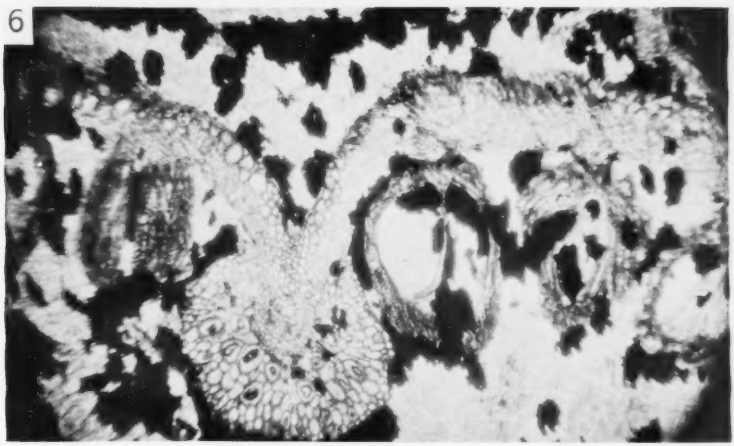


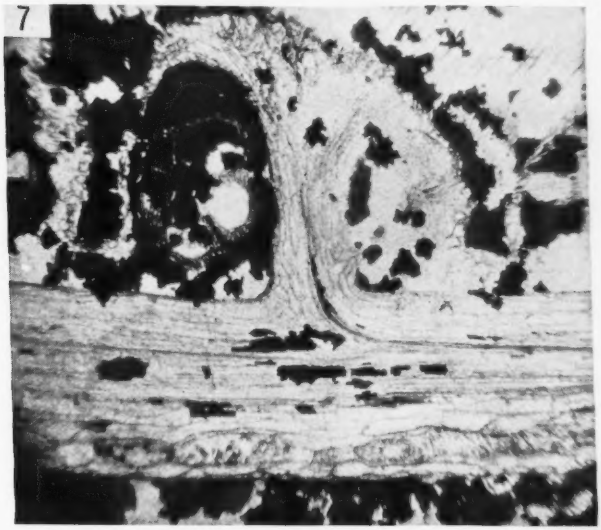








HOSKINS on PENNSYLVANIAN PLANTS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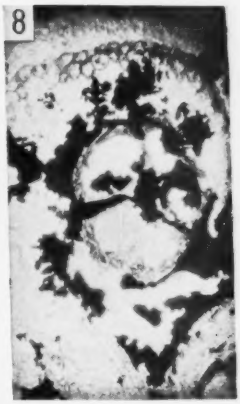
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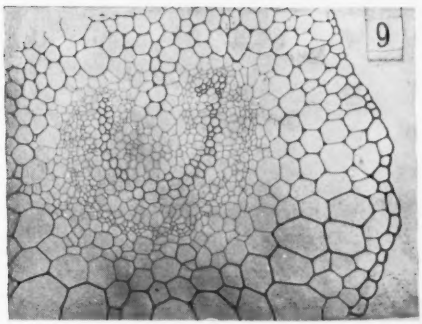
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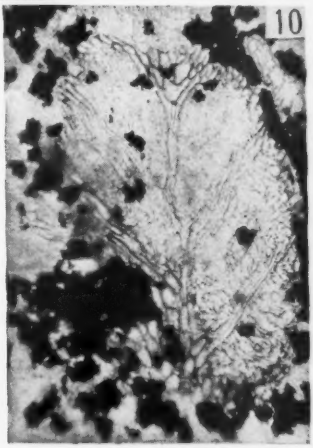
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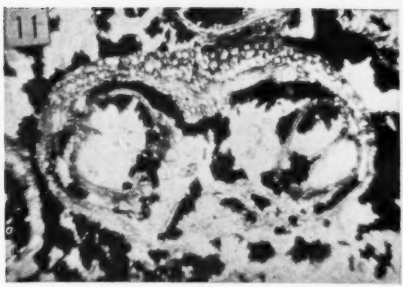
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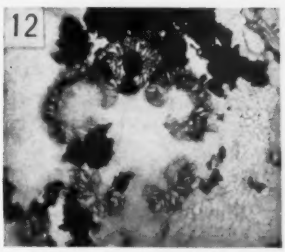
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EXPLANATION OF PLATES XXIII, XXIV

PLATE XXIII

FIG. 1.—Transverse section of ultimate rachis, with characteristic horse-shoe-shaped vascular bundle; ×35.

FIG. 2.—Section parallel to surface of pinnule: to right of midvein are two sori in transverse section, one with four sporangia and one with five; ×16.

FIG. 3.—Transverse section of portion of pinnule showing furled edge of pinnule and attachment of sorus; ×20.

FIG. 4.—Oblique section through ultimate rachis with pinnule cut parallel to surface, showing venation and one sorus in transverse section; ×16.

FIG. 5.—Longitudinal section of sorus showing parts of three sporangia and method of attachment of sorus; ×20.

FIG. 6.—Transverse section of ultimate rachis through two pinnules, parts of four sori shown; ×35.

PLATE XXIV

FIG. 7.—Longitudinal section of ultimate rachis with one pinnule attached, showing single pinnule trace clearly; to left of midvein of pinnule is sorus in transverse section, in lower sporangium of which are spores; ×35.

FIG. 8.—Transverse section of sorus of five sporangia; ×27.

FIG. 9.—Camera lucida drawing of fig. 1.

FIG. 10.—Section of pinnule in plane of surface, showing type of venation.

FIG. 11.—Transverse section of pinnule: edges of pinnule curved, partially inclosing the two sori; common attachment of each sorus evident; ×27.

FIG. 12.—Transverse section of sorus near distal ends of sporangia; dehiscence occurred through longitudinal slit on inner surface of each sporangium; ×25.

LEGUME INOCULATION AS INFLUENCED BY STOCK AND SCION

THOMAS E. RICHMOND

(WITH TWO FIGURES)

Introduction

That the nodule bacteria of *Phaseolus lunatus* are distinct from those of *P. vulgaris* has been shown by WHITING and HANSEN.[1] This probably is the first case on record in which all the species within a given plant genus are not inoculated by the same nodule organism. As yet no fundamental relationship has been reached upon which a satisfactory grouping of the nodule organism for inoculation purposes can be made.

As it is possible to grow to maturity a lima bean top on a navy bean root, or a navy top on a lima root, an entirely untouched method of investigating the relation of the host plant to its nodule-producing organism is opened for study. The answer to such questions as follows might throw light upon the relationship of the host plant to its nodule organism: (1) Which organism, that specific to the lima or to the navy bean plant, will produce nodules upon a navy root when grafted with a lima top? (2) Will the nitrogen content of a lima top upon an inoculated navy root be the same as that of a normally inoculated lima plant? (3) Will the processes of photosynthesis and metabolism be the same in a grafted plant as in the normal plant? (4) Will the nitrogen compounds found in the seeds from uninoculated, normally inoculated, and grafted plants be the same? (5) Will the seeds produced by grafted plants produce plants that still retain the power of selection between the nodule organisms?

Preliminary work

In most of the following work sterilized seeds were planted in quartz sand and allowed to grow to a height of 6–8 inches before washing out and making the desired grafts. Grafting was performed

[1] WHITING, A. L., and HANSEN, R., Cross inoculation studies with the nodule bacteria of lima beans, navy beans, cowpeas, and others of the cowpea group. Soil Science 10: no. 1. 1920.

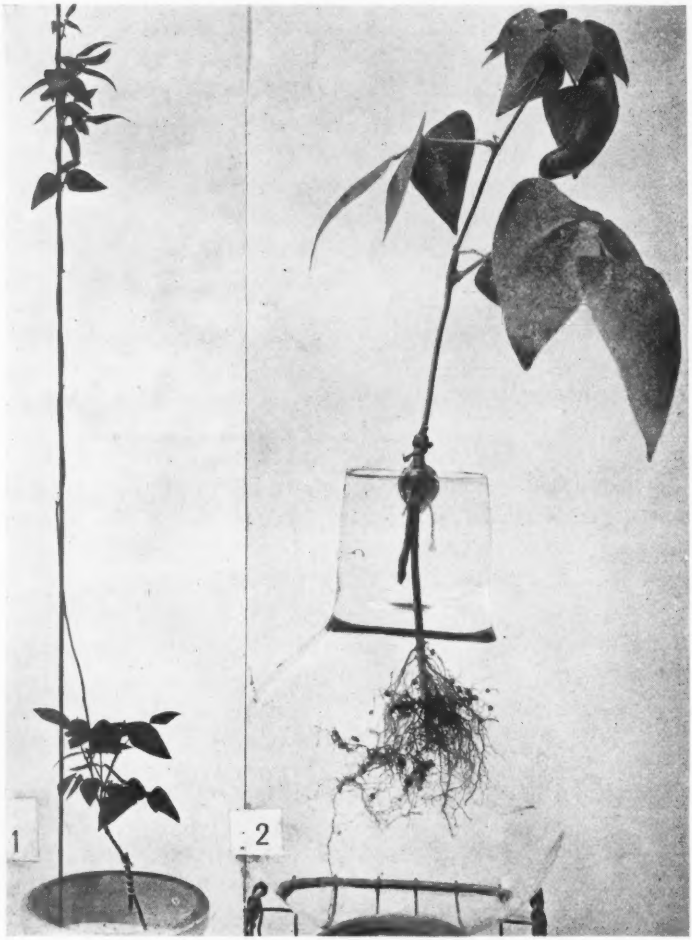
by cutting half the stem away for a distance of about 4 inches. Lima and navy seedlings thus treated were placed so that the two cut surfaces were together and bound firmly to each other with string. After the seedlings were securely fastened, the united stem was punctured in several places with a needle. The combined plants were then replanted in quartz sand in a gallon jar and tied to a support. In about a week's time the stem of one of the two beans was nearly cut in two between the root and bottom of the graft, and at the same time the top of the other plant was cut off. In a few days' time the cut root was severed and removed from the jar. This would then leave a lima bean root with a navy bean top, or a navy root with a lima bean top, as the graft happened to be made. In all cases the plants were watered with tap water, which was found to be not only free of the nodule organism, but also contained sufficient plant food elements, with the exception of nitrogen, to grow plants to maturity. After a successful graft had been made, the nodule organism to be used was added to the jar and the plant allowed to grow for the required time.

EXPERIMENT 1.—The first graft made was a lima top upon a navy root. This was successful and the nodule organisms of the lima bean added to the jar June 1, 1924. Planted in this same jar were sterilized seeds of both lima and navy beans, which were allowed to grow as normal plants in order to test the purity of the nodule organism used. The grafted plant grew well, and upon examination of the check plants in July it was found that the navy bean was uninoculated while the lima bean was well inoculated. This indicated that the lima bean culture used caused nodulation upon lima, but not upon navy bean, and was therefore a pure culture of the lima bean organism.

The grafted plant grew to a height of several feet, and produced well developed pods with several beans in each (fig. 1). This plant was washed out September 2, 1924, and it was found that the root, while free from nodules, had been stimulated to a marked degree. The dry weight and total nitrogen determination for this plant were as follows:

Oven-dry weight of top (except seeds) 2.40 gm.
Oven-dry weight of roots.....1.62 gm.
Weight of nitrogen in top .....49 mg.
Weight of nitrogen in roots.....34 mg.

As this plant had matured several beans, and still had a nitrogen content of 83 mg., the fact is evident that it must have been able to obtain nitrogen even though no nodules were formed upon its roots.



FIGS. 1, 2.—Fig. 1, lima bean top-navy bean root, treated with lima bean nodule organism; leaves removed from part of stem; fig. 2, lima bean top growing upon an inoculated navy bean root.

The nitrogen content of the original bean, as determined by the analysis of similar beans, was about 12–15 mg. The normal navy bean plant grown in the same jar, as a check upon the organisms used, was dying of nitrogen starvation when removed for examination of its roots for nodules, showing that the nitrogen added in the bacterial culture and tap water was not the source of nitrogen available to the grafted plant.

EXPERIMENT 2.—In this trial, grafted plants were inoculated with the organism common to the root of the graft, and it was found in every case in which the navy bean organism was added to a jar in which a lima top was growing upon a navy root, that inoculation occurred and the grafted plant grew to maturity and produced seed. The reciprocal grafts and inoculations with lima bean cultures also produced nodules in all cases.

EXPERIMENT 3.—In this case a lima bean top was grafted upon a navy bean root that already had nodules upon its roots. This graft was successful and the plant grew well. The graft was made June 1, and washed out August 8, 1924 (fig. 2). The plant had several mature beans and was normal in all respects. The root was in good shape and the nodules active. This lima bean top had been able to obtain nitrogen through the inoculated navy bean root.

EXPERIMENT 4.—The question of the behavior of beans grown upon grafted plants toward the nodule organism was next investigated. Navy beans which had grown upon lima roots, and lima beans which had been grown upon navy roots were used in this experiment. The sterilized beans were planted in quartz sand and inoculated June 9, 1924, as indicated:

	Nodulation
Modified navy bean seed plus navy bean bacteria	+
Modified navy bean seed plus lima bean bacteria	+
Modified lima bean seed plus navy bean bacteria	+
Modified lima bean seed plus lima bean bacteria	+
Normal lima bean seed plus navy bean bacteria	−
Normal navy bean seed plus lima bean bacteria	−

These plants all grew well and were washed out July 18. Nodules were found upon the roots of all the plants except the checks, but it was at once apparent that the nodules upon the navy bean roots inoculated with lima bean organisms and those upon the lima beans

inoculated with navy bean organisms were quite different from normal nodules. They occurred only upon the main root, which appeared to be enlarged and cracked at the point of union, and were whitish in color and flat. The plants, however, gave no appearance of nitrogen starvation.

As normal lima and navy beans inoculated and grown in this same way gave no signs of nodule formation, it appears that the beans grown upon grafted plants have in some way been modified so that they are no longer able to differentiate between the nodule organisms of the lima and navy bean.

Summary

1. When a lima bean top is growing upon a navy bean root, in quartz sand, a pure culture of lima bean bacteria will not cause the formation of nodules upon the roots of the grafted plant, but the roots are stimulated, the plant grows to maturity, and appears to be able to obtain atmospheric nitrogen.

2. When such a graft is inoculated with a navy bean culture, nodules are formed and the plant grows to maturity. The navy bean organisms in the roots apparently are able to furnish nitrogenous compounds to the lima bean tops, in exchange for carbohydrates synthesized by a top not normal to the nodule organism used.

3. Similar results are obtained with the reciprocal grafts and inoculations.

4. A lima bean top grafted upon an inoculated navy bean root grew to maturity and developed seeds.

5. When seeds are produced by a grafted plant, either lima or navy, they are so modified that plants grown from such seeds no longer have the power of selective adaptation for the specific nodule organism common to it, but are inoculated by either the lima or navy bean organism.

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CURRENT LITERATURE

BOOK REVIEWS

Manual of plant diseases

A wealth of well selected and judiciously arranged material is packed into HEALD'S new volume on plant diseases.[1] It will be useful to the student primarily as a reference book, since treatment of the material in the main is encyclopedic. As a starting point in dealing with various non-parasitic diseases, the author briefly discusses the requirements and reactions of the normal or healthy plant. This introduction of some of the fundamental principles of the subject greatly enhances the value of the work as a text. A considerable part of the value of the book for reference lies in the accurate and up-to-date presentation of the material, the painstaking reference to sources for statements, and the excellent bibliographies which close each chapter. Illustrations and diagrams are well selected and well done, and increase the utility of the volume.

In defining the field of plant pathology the author refers to phytopathologists as "the trained plant doctors, the 'medicine men of agriculture,' whose final goal is successfully to prevent or control plant diseases." This statement sounds the keynote of the book. The material is presented from the point of view of training the "farmer, gardener, or professional plant doctor" to make diagnoses and to employ or recommend proper control measures. It would have been well to point out that the field of phytopathology also includes investigations in the fundamental biological aspects of the subject, such as the phenomena of pathological anatomy, parasitism, and immunity, for the history of phytopathology parallels that of medicine, in that, in the main, like medicine it is founded not on empiricism but on scientific investigation, much of which has its origin in pure intellectual curiosity. Even if we ignore this failure to point out the real field of the science of phytopathology as contrasted with its art, the comparison is unfortunate. Whatever success the plant pathologist has had in controlling plant diseases has been by prevention rather than by cure or medication. The method and goal of the phytopathologist are more akin to those of the worker in public health than to those of the medicine man.

By including the pathological conditions of plants due to non-living factors (non-parasitic diseases), the author departs from the usage of the older American writers on plant pathology. This is a decided gain. The book does not go all the way, however, in defining the field of phytopathology. The only reference to the pathological conditions of plants induced by animals, save that due to nematodes, is an attempt to justify the present usage of assigning

[1] HEALD, F. DEF., Manual of plant diseases. 8vo. xiii+891. *figs. 272*. New York: McGraw Hill Book Co. 1926. $7.00.

the field to the economic zoologist and entomologist who are only secondarily interested in the affected plant. Nine chapters (176 pages) are devoted to non-parasitic diseases; one chapter (55 pages) to virus diseases; thirteen chapters (502 pages) to diseases due to the activity of bacteria and fungi; one chapter (21 pages) to pathological conditions induced by parasitic seed plants; one chapter (22 pages) to diseases induced by nematodes; and one chapter (20 pages) to the structure of fungi with a slight reference to their physiology. The use of such phrases as "Pondscum parasites" instead of Chytridiales, or "Black molds and allies" instead of Zygomycetes in chapter captions has nothing to commend it, since the manual was not designed for popular appeal.

The book is well printed and illustrated, and should go a long way toward meeting the demand for a modern text of plant pathology.—G. K. K. LINK.

Gymnosperms

The second edition of the gymnosperm section of ENGLER and PRANTL's *Die Natürlichen Pflanzenfamilien*, just issued by ENGLER,[2] is much enlarged and improved, with the 127 pages and 82 figures of the first edition increased to 441 pages and 240 figures. In the first edition only four families, the Cycadaceae, Cordaitaceae, Coniferae, and Gnetaceae were recognized; while in the second edition the material is treated under seven orders: Cycadofilices, Cycadales, Bennettitales, Ginkgoales, Cordaitales, Coniferae, and Gnetales. PILGER supervised the whole work and wrote the living Cycadales, Ginkgoales, and Coniferae, while KRAÜSEL added the fossil members of these orders. GOTHAN wrote the Cycadofilices, and MARKGRAF the Gnetales.

The family ending "aceae" is used in the Coniferae, so that we have Cupressaceae instead of Cupressineae, etc., and a similar raising of rank appears in other places. It is to be regretted that in a work which is so widely used, there is so little consistency in the endings. The ending "ales" has become almost as well recognized as an ordinal ending as "aceae" is for a family ending. Cycadofilicales and Coniferales would have made the series consistent.

In the Cycadales the number of genera remains 9 and the number of species about 83; but the number of pages dealing with this group has been doubled. The treatment, even from the morphological standpoint, reflects much of the progress of the past 30 years. The keys, which in the old edition were based upon cone characters, are here based primarily upon vegetative characters, which make them more serviceable, because cycads usually are not in fruit. Vegetative characters could have been used to a still greater extent.

The greatest changes and largest additions have been made in the Coniferales, which now have 286 pages instead of 88, and 107 illustrations instead of 58. Seven families are recognized, the Taxaceae, Podocarpaceae, Araucariaceae, Cephalotaxaceae, Pinaceae (including Abietoideae and Pinoideae),

[2] ENGLER, A., Die Natürlichen Pflanzenfamilien. Gymnospermae. pp. 447. *figs.* 240. Leipzig: Wilhelm Engelmann. 1926.

Taxodiaceae, and Cupressaceae; a very different sequence from that of the old edition, which began with the Pinoideae and ended with the Taxeae. The Cupressaceae are probably a very advanced group, but many will not approve the position assigned to the Taxaceae or the placing of the Araucariaceae between two taxad groups.

While primarily the volume is a taxonomic work, a large proportion of the space is given to morphology, phylogeny, and geographic distribution, and the lists of literature contain many references to these phases of the subject.

One of the most important improvements is the rather full account of extinct forms, which received a very inadequate treatment in the old edition. Many of the illustrations are new, and some of those in the Cycadales are taken from SCHUSTER'S forthcoming monograph of this order.—C. J. CHAMBERLAIN.

Ion effects in colloidal systems

All biologists will welcome the publication of the little volume by MICHAELIS[3] embodying lectures delivered in 1924 at various universities in the United States. Most of the book is taken up with a discussion of adsorption and electrical double layers, with emphasis on the importance of a correct understanding of these, if the biologist is to understand the life processes of the cell. The author considers of course that the condition of the colloidal material of the cell controls its vital functions, for example, respiration, irritability, etc. The condition of the colloidal material depends upon its electrical charges, however, hence the importance of a correct understanding of electrical double layers and adsorption.

The volume opens with a statement of the author's intention in regard to the use of the term adsorption. The term is used to include not only cases of real chemical union, that is, where the compound adsorbed is molecularly combined with the adsorbing medium, but also the cases where the union is more physical, the compound adsorbed accumulating in the more superficial layers of the water. Chapter II takes up certain electrical phenomena resulting from the adsorption of ions. Electromotive forces, cataphoresis, and endosmosis are discussed. Next is considered the origin of electrical double layers. Three possibilities are discussed, double layers resulting from appositional adsorption, those formed as a result of the dissociation of colloidal particles, and those formed at the surface of chemically inert materials such as collodion, cellulose, etc. There follows a discussion of the adsorption of ions by charcoal, and a consideration of the quite peculiar place held by charcoal as an adsorbing medium, this being mainly due to the fact that, in contrast with most substances, it has a great power of adsorbing non-ionized, capillary active compounds. The analogy of this peculiar property to conditions in the living cell is discussed. Next is considered the Donnan equilibrium. The author reconciles the production of an electrical double layer in this phenomenon with the other types of

[3] MICHAELIS, L., The effects of ions in colloidal systems. pp. 108. Baltimore: Williams and Wilkins Co. 1925.

electrical double layers by regarding it as belonging to the case of the production of an electrical double layer by the dissociation of colloidal particles. The last two chapters consider the lyotropic effects of ions, and the effects of mixtures of ions.

The discussion is compact, and one needs to have done considerable reading on the points taken up fully to understand them. The author fully appreciates the insufficient state of our knowledge of adsorption and electrical double layers. There is a proper balance between the statement of facts as revealed by experiments and the theoretical part, which looks ahead and calls for further experimentation. The book should be very helpful to biologists, and should stimulate further research in this important and difficult field.—S. V. EATON.

Plant nutrition and crop production

The Hitchcock Lectures at the University of California in 1924 were given by Sir JOHN RUSSELL, the distinguished director of the Rothamsted Station. These lectures have been published in book form,[4] and are worth possessing by anyone interested in any way in the scientific side of plant nutrition and the production of crop plants. There are five lectures, the first of which deals mainly with the background of history of the study of plant nutrients. Naturally this follows along the same lines as the historical account in his previous book on *Soil conditions and plant growth*.

The second chapter is entitled "Positive science and exact demonstration." It emphasizes the value of quantitative studies of plant behavior to soil factors and other influential environmental conditions. The reference to auximones does not fit in so well with the title of the chapter, as it is not yet demonstrated that there is such a thing as an auximone. BOTTOMLEY'S results have failed of confirmation in a number of studies, and it is certain that his original cultures were poorly balanced as to minerals. Likewise, the auximones without doubt contained balancing minerals. The third lecture discusses decay and the living plant, and considers the function of organic matter in the soil and some of the organisms occurring there, and particularly the relations of bacteria and Dimastigamoebae. The fourth discourse concerns the question as to whether the microorganic population can be controlled or utilized. Inoculation, sterilization, and control through favoring the helpful and discouraging the harmful forms of living things are excellently discussed. The final chapter deals with the great complexity of the relations of plants as organisms with the soil, itself enormously complex.

The book is written in a charming style, and the 21 plates and 37 figures give it an unusually fine appearance. The plate showing pictures of GILBERT, LAWES, LIEBIG, and BOUSSINGAULT will be appreciated by all possessors of the volume. It is in every way an excellent work which should have a wide distribution.—C. A. SHULL.

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[4] RUSSELL, SIR JOHN, Plant nutrition and crop production. 8vo. pp. x+115. University of California Press. 1926. $2.50.

The Aspergilli

A much needed book on the the genus *Aspergillus* has been written by THOM and Miss CHURCH.[5] The book has grown out of the extensive laboratory work with members of the genus *Aspergillus* begun by THOM in 1904, when he undertook investigation of the molds which play a rôle in food handling. In the course of their studies, the authors examined the original description of all save three or four of the sixty odd species of *Aspergillus* listed in the book. The book will be extremely useful to workers in many fields. Some Aspergilli are favorites for biochemical studies; some play an important rôle in industrial fermentations; some cause disease of animals; some play an imporatnt rôle in the spoilage of fruits, vegetables, and nuts in transit and in storage; and finally, many of them play a provokingly persistent and important rôle as "weeds of the culture room." A history of *Aspergillus* is followed by discussion of its morphology, physiology, biochemistry, and taxonomy. There are three useful keys: one based on color of the heads and stalks, the second a synoptical key based upon actual cultures of Aspergilli, and the third an abbreviated form of the second. Separate chapters are devoted to such topics as Culture of Aspergilli; Physiological and Biochemical studies; Enzymic and fermentative activity of Aspergilli and their Industrial significance; and Pathogenic aspects.—G. K. K. LINK.

British lichens

All students of the lichens in America or elsewhere make constant use of SMITH'S admirable works on the lichens of Great Britain. The first part of the first edition was the work of Rev. JAMES CROMBIE, but after his death in 1906 the task was taken over by ANNIE LORRAIN SMITH, who issued the second part of the first edition in 1911. The first part of the second edition appeared in 1918, and the concluding (second) part of this edition has now appeared.[6] The author in 1921 also published her extremely useful *Handbook of British lichens*, which has had very large use by students of lichens here and abroad.

The new edition shows no fundamental changes, although there are some alterations in the grouping of species, especially in *Lecidea*. The present volume contains descriptions and habitat data concerning some of the Cyclocarpineae not considered in the first part, especially the Lecideaceae. There are also treated here the Graphidineae and the Pyrenocarpeae. In the latter pages is a list of microfungi recorded by British authors as lichens, also an appendix to Part I with emendations and additions. The work concludes with a most admirable glossary of terms used in lichenology, and an index which includes not only generic but also specific names and synonyms.—H. C. COWLES.

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[5] THOM, C., and CHURCH, MARGARET B., The Aspergilli. 8vo. pp. ix+272. *figs. 13. pls. IV.* Baltimore: Williams & Wilkins Co. 1926. $5.00.

[6] SMITH, ANNIE LORRAIN, A monograph of the British lichens. A descriptive catalogue of the species in the Department of Botany, British Museum. Part II. 2d ed. revised. pp. ix+447. *pls. 63.* London: British Museum (Nat. Hist.). 1926.

A naturalist in East Africa

An interesting account of his travels in Uganda has been published in book form by CARPENTER.[7] The author, who was medical officer at various military posts, spent his leisure in studying the natural history of the region. Particular attention was given to the insect life, problems of protective coloration being among the topics discussed, and brief notes on the vegetation are also recorded. The excellent illustrations range from photographs of butterflies, giant lobelias, and bamboo forests, to views of Victoria Falls.—G. D. FULLER.

NOTES FOR STUDENTS

Functions of transpiration.—Various functions for transpiration have been given from time to time. By some it has been considered a process essentially useless or even injurious to the plant, because of the large amount of water lost in the process. At the other extreme are those who consider transpiration a process very beneficial; in fact, a process without which the plant could not live. Some of those who hold this view speak of two main benefits accruing to the plant from transpiration, the cooling effect of the process, and the importance of the process in the transfer of salts into the plant and from the roots to the growing parts.

CURTIS[8] has given an excellent summary of the significance of transpiration. As to the cooling effect of transpiration, reference is made to several papers, among them a paper by CLUM,[9] showing that the temperature of the leaf is reduced by transpiration not more than from 1° to 5° C. CLUM vaselined certain leaves and compared the temperature of these with unvaselined leaves. The leaves in which transpiration was checked by vaseline were only 2°–4° C. warmer than the unvaselined leaves. In another paper, CLUM[10] shows that the temperature of a leaf may be reduced as much as 7° C. by shading it, thus illustrating the efficiency of convection and radiation in cooling the leaf. CURTIS and CLUM regard light intensity, angle of incidence, convection and radiation, and air currents as having a much greater effect on the temperature of the leaf than the cooling effect of transpiration. It may well be that the loss of heat by conduction and convection is rapid enough to prevent undue heating when transpiration is checked; but, in view of the large amount of water transpired by plants and the high heat of vaporization of water, it would seem that under

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[7] CARPENTER, G. D. H., A naturalist in East Africa. 8vo. pp. 187. *pls. 8. figs. 23.* 3 maps. Oxford Univ. Press; American branch, New York. 1925. $5.

[8] CURTIS, O. F., What is the significance of transpiration? Science **63**:267–271. 1926.

[9] CLUM, H. H., The effect of transpiration and environmental factors on leaf temperatures. I. Transpiration. Amer. Jour. Bot. **13**:194–216. 1926.

[10] ———, II. Light intensity and the relation of transpiration to the thermal death point. *ibid.* 217–230. 1926.

normal conditions, when transpiration is not checked, it accounts for the dissipation of much of the solar energy absorbed by plants. BROWN and ESCOMBE's balance sheet of the solar energy falling on the leaf certainly gives an important place to transpiration in the dissipation of this energy. According to their viewpoint, transpiration would not be a necessary process for keeping down the temperature of the leaf, for when transpiration is checked, conduction and convection cool the leaf, but this does not alter the fact that under normal conditions transpiration may account for the use made by the leaf of much of the energy absorbed by it.

In regard to the importance of transpiration in relation to the movement of salts into the root, and from the root to the growing parts of the plant, reference is made to work of MUENSCHER showing that high transpiration does not cause increased movement into the plant and from the roots to the tops. While salts certainly enter the root from the soil solution and pass through the cortical cells of the root to the tracheae independently of the water, yet, as CURTIS points out, if the salts pass by mass movement in the transpiration stream from the roots to the leaves, it is hard to see why this does not cause increased absorption by the root and therefore a concentration of salts in the tops. On the other hand, if the salts are not carried along by the transpiration stream from the roots to the leaves, it is hard to see how the growing parts of the plant are provided with enough salt material.

Other points included by CURTIS in his summary are effects in the plant of a reduction of the water content of the plant, effects of a reduction of the water content of the soil, and certain effects that are often falsely ascribed to transpiration.

As CURTIS points out, most of the work on transpiration has had to do with the amount of water transpired, and the factors affecting this water loss. Much more experimental work is needed on the functions of transpiration. CURTIS has done a big service in again opening up this subject.—S. V. EATON.

Taxonomic notes.—In his fourth paper on "new and noteworthy fungi," DEARNESS[11] has published 9 new species of Discomycetes in 5 genera, 18 new species of Pyrenomycetes in 15 genera, one of which (*Phragmodothidea*) is new, and 12 new species of Deuteromycetes in 9 genera.

Miss WEEDON,[12] in publishing the results of her collection of fungi near St. Petersburg, Florida, in addition to 4 new species, has described 2 new genera, *Exophoma* on *Magnolia*, and *Macrophomopsis* on *Dracaena*.

In continuation of his revision of the grasses of Japan, HONDA[13] has described 7 new species, 4 of them belonging to *Calamagrostis*.

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[11] DEARNESS, JOHN, New and noteworthy fungi. IV Mycologia **18**:236–255. 1926.

[12] WEEDON, AMY G., Some Florida fungi. Mycologia **18**:218–223. 1926.

[13] HONDA, M., Revisio Graminum Japoniae. X. Bot. Mag. Tokyo **40**:317–327. 1926.

KOIDZUMI[14] is publishing a series of contributions to the flora of eastern Asia, the knowledge of which is increasing rapidly. In the present paper, among the numerous additions to this flora, there are described new species of *Fraxinus*, *Salix*, and *Hydrangea*.

TIFFANY[15] has published the results of collections of filamentous algae made in northwestern Iowa during four seasons. It is a region of lakes, swamps, and streams that are rich in freshwater algae. The report includes 35 Myxophyceae and 165 Chlorophyceae, by far the largest genus being *Oedogonium*, with 68 species, one of which is described as new. The majority of the forms have not been reported for Iowa, and 6 forms of *Spirogyra*, 15 of *Oedogonium*, and 3 of *Bulbochaete* have not been recorded previously from North America.

MERRILL[16] has published a third paper recording additions to the Philippine flora. The species considered are mainly from the Sulu Archipelago, from two islands that had never been explored botanically. The paper lists 47 species as new to the Philippine flora, 32 of which, distributed among 30 genera, are described as new. Eight of the genera are recorded for the first time from the Archipelago.

JACKSON[17] has begun the publication of the collections of rusts made in South America by E. W. D. HOLWAY during two excursions, which included Ecuador, Bolivia, Peru, Chile, Brazil, and Argentina. The rusts are presented by families of the host plants, representing 49 species on 71 hosts, 13 of the species being described as new.

PERCIVAL[18] has described 21 new varieties of emmer wheat obtained from Abyssinia, Somaliland, Morocco, and Transcaucasia, grown in the highlands of the country at an elevation of about 10,000 feet. In the same paper there are described also 7 new varieties from regions of Asia Minor.

HANSFORD[19] has published a very full account of the genus *Fusarium* as found in Jamaica. The work was done in connection with experimental work on the Panama disease of the banana, which necessitated an examination of the *Fusarium* flora of a large number of soil samples. As a result, 35 species and varieties are recognized and classified.

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[14] KOIDZUMI, G., Contributiones ad cognitionem Florae Asiae Orientalis. Bot. Mag. Tokyo **40**:330–348. 1926.

[15] TIFFANY, L. H., The filamentous algae of northwestern Iowa, with special reference to the Oedogoniaceae. Trans. Amer. Micr. Soc. **45**:69–132. 1926.

[16] MERRILL, E. D., Additions to our knowledge of the Philippine Flora. III. Phil. Jour. Sci. **30**:389–430. 1926.

[17] JACKSON, H. S., The rusts of South America based on the Holway collections. I. Mycologia **18**:139–162. 1926.

[18] PERCIVAL, J., Some new varieties of wheat. Jour. Bot. **64**:203–210. 1926.

[19] HANSFORD, C. G., The Fusaria of Jamaica. Kew Bull. Miscell. Inf. no. 7. 1926.

PIPER[20] has published the results of his studies of certain groups of Phaseolineae (Fabaceae). Twelve genera are presented, two of which are new (*Condylostylis* and *Alepidocalyx*). Much the largest genus is *Phaseolus*, with 95 species, 36 of which are described as new.

SMALL[21] has described a new species of *Zamia* (*Z. silvicola*) from "peninsular Florida," said to be the most robust *Zamia* in Florida.

CURTIS[22] has described a new genus (*Claustula*) of Phalloid Fungi, which he collected in New Zealand. The systematic relationship is not clear, since sufficiently young material to trace the early development was not available. The author's conclusion is that "the fungus is either an unusual member of the Phallineae or has affinities with that group."—J. M. C.

Artificial selectively semipermeable membranes.—Selective semipermeability is well known among plant membranes, both living and lifeless, but the causes of selective behavior have never been fully explained. The production of artificial membranes which duplicate the type of behavior of seed coats and plasmatic membranes is a rather remarkable achievement. KAHLENBERG has been interested in permeability and osmosis for many years, and has now[23] been able by dialysis to separate crystalloids from one another. He even claims that it is possible to separate colloids from crystalloids by having the colloids pass through the dialyzing membranes, leaving the crystalloids behind, by proper choice of septa and solvents.

For nonaqueous solutions the septum used is rubber damask and the solvent pyridine, which is unique in being miscible in all proportions in water, and at the same time equally miscible with other organic solvents which do not mix with water. Sulphur, naphthalene, and camphor, for instance, all dissolve in pyridine, and also in hydrocarbons. Since rubber behaves like a hydrocarbon, these substances in solution in pyridine will pass the rubber septum into pure pyridine on the opposite side of the membrane. Silver nitrate, lithium chloride, and sucrose, although soluble in pyridine, will not dissolve in hydrocarbons, and will not penetrate rubber. It follows, then, that a solution of sulphur and lithium chloride in pyridine can be separated by dialysis through the rubber membrane, sulphur passing and leaving the lithium salt behind. All of the substances insoluble in rubber can be removed quantitatively from those not soluble in rubber in this way, in from two days to three weeks.

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[20] PIPER, C. V., Studies in American Phaesolineae. Contrib. U.S. Nat. Herb. **22**: 663–701. 1926.

[21] SMALL, J. K., Cycads. Jour. N.Y. Bot. Gard. **27**:121–129. 1926.

[22] CURTIS, K. M., The morphology of *Claustula Fischeri*, gen. et sp. nov. A new genus of Phalloid affinity. Ann. Botany **40**:471–477. 1926.

[23] KAHLENBERG, L., On the separation of crystalloids from one another by dialysis. Philosophical Mag. (7th series) **1**:385–394. 1926.

For separation of solutes in aqueous solution a membrane of lanoline supported by parchment paper or on a fine quality of China silk was used. They were made by soaking the silk in a chloroform solution of lanoline. Collodion membranes were impracticable as a basis for lanoline films, although they can be made. Pieces of lanoline silk tied over thistle tubes in the usual way were used to separate crystalloids by dialysis. Cane sugar, lactose, and dextrose were found not to pass even in two weeks' time, while urea, boric acid, and sodium chloride went through more readily. The speed of passage of urea and sodium chloride differed so much, however, that these were separated fairly well by dialysis. Hydrochloric acid required seven days to pass perceptibly. Through these membranes the author reports separation of urea from mannose, the latter substance not passing through the membrane until after 31 days. Sodium chloride was separated from nickel chloride, boric acid from cane sugar, and urea from sodium chloride.

The ingredient of lanoline responsible for this behavior is found to be cholesterol, and the plant phytosterols were found to possess the same properties. The idea proposed is that "it is extremely delicate films of these sterenes that give living cells their wonderfully selective osmotic properties." No other waxy constituents were found capable of giving these properties to films of silk or parchment paper. The hypothesis is worthy of consideration in connection with plasmatic membrane behavior, and the properties of seed coats. It must be remembered, however, that a cell which did not allow sugar to pass through its membranes for a month would find it hard to secure the needed supplies of glucose for respiration. The plasmatic membranes are permeable to all the salts and organic nutrients used in metabolism.—C. A. SHULL.

Structure of chloroplast.—For a correct understanding of the basic process, photosynthesis, it is important of course to understand the structure of the chloroplast. Much work has been done in an attempt to make clear this structure, but there are still many points about which very conflicting views are held. ZIRKLE[24] has made a thorough study of the chloroplast, and points out that the ordinary methods of fixing, staining, and sectioning do not give a correct understanding of the structure of the chloroplast, because the reagents dissolve out the pigments and alter the state of the stroma. Also, the pigments interfere with any attempt to study the stroma directly in living chloroplasts. ZIRKLE studied the living chloroplasts in monochromatic light. By using light of a wave length corresponding with the chief absorption band of a pigment, this pigment appeared black in transmitted light, and any pigment not absorbing this wave length was invisible. Thus, by optical means which did not alter the state of the stroma or the pigments, he was able to get rid of the pigments and study the stroma in its natural condition. He could also determine the

[24] ZIRKLE, CONWAY, The structure of the chloroplast in higher plants. Parts I and II. Amer. Jour. Bot. **13**:301–320; 321–341. 1926.

distribution of the pigments in the stroma. The inclusions of the chloroplast were studied in polarized light. For the purpose of making certain chemical tests on the chloroplasts, they were extruded into a fluid of about the same osmotic concentration, hydrogen-ion concentration, and viscosity as the cytoplasm of the cells.

ZIRKLE states that the chloroplast is a hollow flattened ellipsoid, having a central vacuole. The starch grain or grains is found in this central vacuole. It has been known for a long time, of course, that there is no sharp distinction between a leucoplast and a chloroplast; that, if exposed to light, a leucoplast may develop chlorophyll and become a chloroplast. ZIRKLE found that some of the chloroplasts of *Elodea* have as their main function the storage of starch rather than its manufacture. In function, therefore, they are leucoplasts, yet they have abundant chlorophyll and are of the same structure as the other chloroplasts. Chloroplasts of seed plants widely separated taxonomically were found to be very similar in structure.

It has long been a question of dispute as to whether there is a real membrane about the chloroplast. ZIRKLE found that there is not a real osmotic membrane. The outer layer of the stroma is somewhat denser than the inner part, and he thinks that this is due to the adsorption on the surface of the stroma of particles of cytoplasm. The apparent granules in the stroma seem to be really pores.

As to the state of the chlorophyll in the chloroplast, ZIRKLE favors the view of WILLSTÄTTER, STOLL, and others that the chlorophyll is in the colloidal state rather than in true solution in lipoids, as LIEBALDT and STERN have concluded. A lipoid solution of chlorophyll is quickly destroyed by light, while ZIRKLE found that extruded chloroplasts of *Elodea* remained green for two weeks. As to whether the chlorophyll is united chemically to the stroma, as LUBIMENKO concluded, or adsorbed on the surface of the stroma, as WILLSTÄTTER and STOLL contended, ZIRKLE, as the result of enzymatic and staining studies, favors the latter viewpoint.

ZIRKLE has done much to elucidate the structure of the chloroplast, and the reviewer feels that the methods used are more likely to give correct information than the methods of many previous workers.—S. V. EATON.

Stomatal movements.—The idea that cells surrounding the guard cells are active in stomatal movements is revived by STRUGGER and WEBER,[25] who have studied the changes in starch content of guard cells, their companion cells, and epidermal cells of *Galium Mollugo* during open and closed condition of the guard cells. They find starch only in the guard cells in closed condition in darkness, and sometimes not even in these. After an hour of illumination, however, the guard cells possess only a trace of starch, while the companion cells contain much of it. The epidermal cells are still otherwise starch free. In several hours of illumi-

[25] STRUGGER, S., and WEBER, F., Zur Physiologie der Stomata-Nebenzellen. Ber. Deutsch. Bot. Gesells. 44: 272–278. 1926.

nation the guard cells are free of starch, the companion cells very full of starch, and the whole general epidermis shows starch storage. They conclude that the correlation betweeen starch amount and stomatal condition must mean that all the cells around a stoma cooperate in the production of movement. Especially the fact that guard cells and companion cells act oppositely with regard to starch content is looked upon as significant. As starch disappears in guard cells it appears in the companion cells, and vice versa. On illumination the starch appears in the companion cells much earlier, more rapidly, and in larger amounts than in the general epidermis.

Osmotic measurements seem to substantiate a relationship. The authors point out, however, that with natural variations in light intensity the stomata undergo extremely rapid changes of stomatal aperture. They think the antagonistic sugar and starch relations of guard cells and companion cells may account for these rapid adjustments. Oddly enough, before midnight, when the stomata are completely closed, the guard cells still may have no starch, a fact noted by others. These latter observations show that the whole hypothesis is faulty.

The rapid closure of stomata has also been noted by SCARTH in a report at the Ithaca Congress. He claims that the rapid responses to light conditions are too quick to be due entirely to hydrolysis of the starch or its condensation. The diastatic action is too slow. Long before the starch undergoes any prominent change the movement is completed. He finds that illuminated and open guard cells are alkaline in reaction, probably from utilization of CO_2 in photosynthesis, and certain colloidal constituents of the cells become much swollen at the P_H developed. High turgidity in the guard cells is mainly caused by the colloidal imbibition. Closure is accompanied by increased hydrogen-ion concentration, due to accumulation of CO_2 of respiration when photosynthesis stops with low light intensity. The colloids are dehydrated as they approach their isoelectric point, the colloidally bound water is freed, the cells quickly lose their surplus water to surrounding cells, and the guard cells close.

If hyperacidity develops due to prolonged darkness, the colloids of the guard cells may become more acid than the isoelectric point, produce an acid swelling of the colloids, and thus bring about night opening of the stomata. Here we have a mechanism whose response is almost instantaneous to light changes, and the sugar and starch reactions may be quite subsidiary to the main regulatory mechanism.—C. A. SHULL.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

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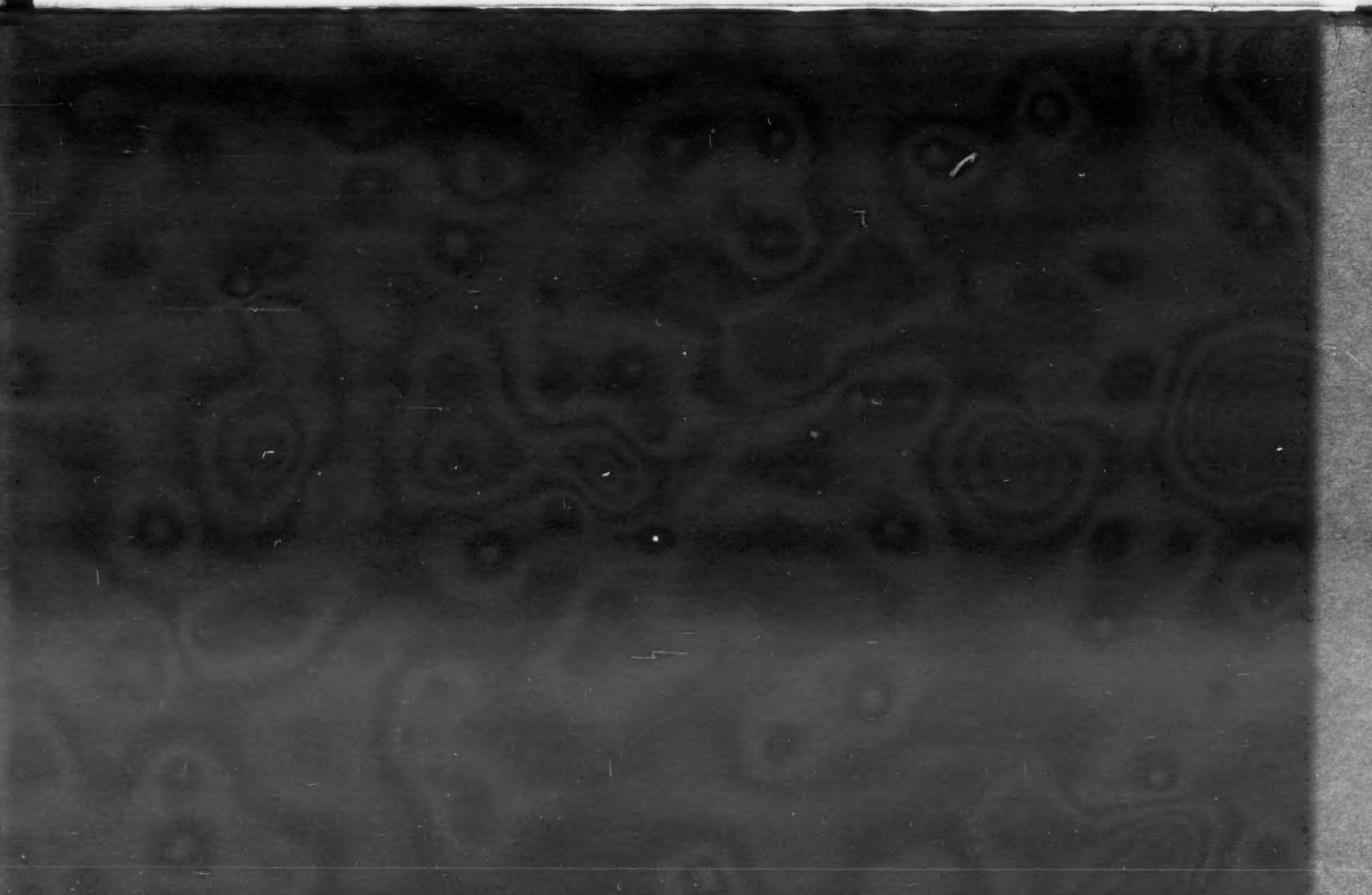

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